Collision avoidance behaviour in a pair of flying locusts (*Locusta migratoria* L.)

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Indika Gayani Benaragama

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ABSTRACT

Migratory locusts; *Locusta migratoria* L. flying in a swarm would encounter spatiotemporally complex visual cues such as translating, receding and looming stimuli, produced by self-motion as well as object motion in the environment. A rapidly approaching conspecific or a predator represents a looming object approaching on a collision course and is involved in triggering urgent collision avoidance behaviours. To avoid predators and collision with conspecifics, and to navigate through complex environments, locusts must produce appropriate collision avoidance manoeuvres. Flying locusts have evolved the ability to not only avoid predation but also effectively navigate within the swarm without constantly colliding with one another. Collision avoidance and predator evasion in response to looming stimuli are important in many animals and in locusts, the key elements in the neuronal pathway underlying this behaviour are the lobula giant movement detector (LGMD) and its postsynaptic component, the descending contralateral movement detector (DCMD). Previous studies have suggested that the LGMD/DCMD pathway allows each locust within a dense swarm to remain sensitive to approaches of individual objects including conspecifics and flying predators, approaching frequently from many directions or along the same trajectory and to produce appropriate collision avoidance behaviours.

Collision avoidance responses of a rigidly tethered locust presented with a looming object have been studied previously. However, behavioural strategies for collision avoidance within a group of conspecifics are yet unknown. Avoidance behaviour exhibited by a single locust may or may not differ from that of an individual in a group. Further, salient cues produced by objects on a collision course (looming) can be influenced by each animal's position relative to the object and/or its position within a group.

In my first objective of this thesis, I exposed locusts (*L. migratoria* L.) to a computer generated looming object in the presence of a live and dead conspecific separately. This first experiment was done to determine if collision avoidance behaviour of a locust: Locust 1 (L1) or Locust 2 (L2), is affected by the presence of a conspecific. As my second objective, the responses of a pair of flying locusts placed in differing relative positions in a wind tunnel were studied during presentation of the same looming object. This second experiment was done to determine if collision avoidance behaviour of a locust is affected by the relative position of a conspecific. From the results, I looked at different spatio-temporal characteristics of L1 and L2 collision avoidance behaviour and their dependency on the presence as well as on different relative positions of a conspecific in the vicinity.

Results from Experiment 1 showed that the types of collision avoidance responses, some components of six degrees of freedom of L1 and L2 and also the timing of the onset and duration of the initial avoidance response of L2, were affected by the presence of a conspecific. According to Experiment 2, the avoidance responses and three translational degrees of freedom of L1 and L2 were also affected by the relative position of the conspecific and its own position, respectively. Also, I found that the timing of the onset and the duration of the initial avoidance response of L2 were affected by its own position in the wind tunnel. Both locusts' responses to the looming stimuli were more robust in the presence of a live conspecific and less pronounced in the presence of a dead locust. Thus, results further suggest that locusts use visual cues from the looming objects as well as an immediate conspecific to generate appropriate avoidance responses. Taken together, the results of my study indicate that a locust's collision avoidance behaviour can be affected by the presence as well as the relative position of a conspecific in the vicinity.

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LIST OF ABBREVIATIONS

LGMD	Lobula giant movement detector
DCMD	Descending contralateral movement detector
3D	Three dimensional
fps	Frames per second
L1	Locust 1
L2	Locust 2
DL	Dead locust
P1	Position 1
P2	Position 2
P3	Front
P4	Back
P5	Up
P6	Down
ANOVA	Analysis of variance

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The migratory locust, *Locusta migratoria* L., is 1 of 12 swarm forming acridid grasshopper species, native to semi-arid regions of equatorial Africa (Uvarov, 1977). It has proven to be an excellent model system in the study of neuroethology (Rind and Simmons, 1997), which is a relatively new scientific study of the neuronal mechanisms involved in adaptive animal behaviours (Zupanc, 2004). Locust swarms may contain up to 10^{10} individuals that travel great distances at approximately 3 ms^{-1} with neighbours flying in the same or different directions, 0.3-9.0 m apart (Uvarov, 1977). Interestingly, despite the apparently random orientation of groups of individuals within the swarm, continuous cohesion of individual swarms over distances of hundreds of kilometres, lasting many days, has been observed to occur without significant dispersion (Baker et al., 1984; Spork and Preiss, 1993). This is because flying locusts have the ability to not only avoid predation but also effectively navigate within the swarm without constantly colliding with one another. The ability to manoeuvre quickly and appropriately in such a dense swarm is of considerable adaptive value (Baker et al., 1981).

To avoid predators, the collision with conspecifics and to navigate through complex environments, appropriate behavioural responses to visual stimuli are essential. The Lobula Giant Movement Detector (LGMD), a looming detector inter-neuron in the locust visual system, functions as the key element of the neural pathway which is implicated in collision avoidance and predator evasion in response to looming stimuli (O'Shea and Williams, 1974; Simmons and Rind, 1992; Judge and Rind, 1997; Gabbiani et al., 2001; Gray et al., 2001; Gray, 2005). Each right and left LGMD synapses onto a single Descending Contralateral

Movement Detector (DCMD) such that 1:1 spiking is maintained via a mixed electrical and chemical synapse (Killmann and Schurmann, 1985). In turn, DCMD conveys information to motor centres in the thorax and excites motor neurons and inter-neurons which are involved in initiating flight avoidance responses and other escape behaviours. The LGMD/DCMD pathway allows each locust within a dense swarm to remain sensitive to approaches of individual objects, including conspecifics and flying predators, approaching frequently from many directions or along the same trajectory (Burrows and Rowell, 1973; Simmons, 1980; Gray, 2005: Guest and Gray, 2006; Santer et al., 2006).

A locust's visual environment consists of a complex combination of translating, receding, and looming visual stimuli that are produced by self motion as well as by object motion (Gray, 2005). In a natural visual scene, a locust will be presented with multiple objects traveling through its visual field along various trajectories (Uvarov, 1977). Collision avoidance responses to looming stimuli are adaptive behaviours that allow the animal to manoeuvre in a complex environment. Preliminary studies with locusts in a wind tunnel have linked this adaptive behaviour with the activity of the LGMD/DCMD pathway (Gray et al., 2001; Santer et al., 2005; Santer et al., 2006; Rind et al., 2008). In these studies, locusts have often been rigidly held in place to facilitate behavioural and physiological recordings. However, the way that a locust responds to a looming object in the presence of other locusts in its visual field when it is free to move in three dimensional (3D) space is unknown. Avoidance behaviour of a single locust may or may not differ from that of an individual in a group. It is possible that collision avoidance responses elicited by an approaching object can be influenced by the presence of a conspecific and each animal's position relative to the looming object and/or its position within the group. As a first step in understanding looming responses of a locust in the presence of conspecifics, I used two locusts simultaneously. In this way, the present study estimated the collision avoidance behaviour of a locust and the

way that a locust's behaviour depends on the presence as well as different relative positions of a conspecific in the vicinity.

1.2 LITERATURE REVIEW

1.2.1 NEUROETHOLOGY

The success of an animal in its particular environment is often related to the animal's ability to gather specific information from multiple sources in its surroundings by the sensory system, to produce appropriate adaptive responses. Integration of the multi-modal information to produce an appropriate behaviour occurs within the animal's nervous system, which integrates environmental inputs and the animal's internal state to generate appropriate motor commands.

Neuroethology is a relatively young science and arguably became a distinct research field in the 1980s. Neuroethology uses evolutionary and comparative approaches to explore the neural connection with the animal's natural behaviour and to uncover the diversity and specialization of nerve cells in the underlying mechanistic control by the nervous system (Zupanc, 2010). However, to reveal a better understanding about the neural control of the behaviour under complex visual contexts, it is necessary to uncover general principles of animal behaviour under more realistic natural conditions.

Neuroethological principles arise from studying various simple, robust, readily accessible and ethologically relevant model systems. Examples include prey capture in the leopard frog (*Rana pipiens)* (Cobas and Arbib, 1992), echolocation in bats (Jones, 2005), pheromone detection in the Oriental silkworm moth (*Bombyx mori*) (Kennedy, 1983), and collision avoidance in locusts. The latter system is the focus of this study.

1.2.2 INSECTS AS NEUROETHOLOGICAL MODELS

Through evolution, animal behaviour has become more complex and this is quite often related to the complexity of the nervous system. Consequently, neuroethologists are challenged with selecting appropriate model systems. Some animal species are well adapted to particular aspects of sensory or motor performance and those superior capabilities are linked to highly specialized neuronal structures. Examples include recognition of prey and predators in toads by neurons in the optic tectum and thalamic pre-tectum, neural systems in electric fishes specialized for time coding (Ewert, 1997; Kawasaki, 2009).

However, some animals have been pressured by natural selection to produce very complex behaviours with relatively 'simple' nervous systems. Insects are good models to study neural mechanisms underlying behaviour since they display many complicated behaviours with a relatively tractable nervous system (Zupanc, 2004). For insects flying in complex, dynamic visual environments, detection of approaching objects such as predators or conspecifics is highly adaptive. Rapid manoeuvrability is essential for individuals traveling at speed in a complex environment when the potential hazards in their visual field are also traveling at speed in different directions, often on a collision course. In this context, an insect's visual and nervous system must be able to detect the approaching objects and elicit appropriate behavioural changes.

1.2.3 LOCUSTS AS A MODEL SYSTEM

1.2.3.1 General biology and locust swarming

Locusts (Fig. 1.1) are acridid grasshoppers which display a polymorphic life history and phase related behaviour. Locusts have the remarkable ability to change between two morphologically, physiologically and behaviourally distinct forms (Uvarov, 1977).

Fig.1.1: Dorsal view of an adult *Locusta migratoria* L. (1x1) (Picture published here with permission from Dr. Igor Grichanov, 2008: Photo © V.V.Neymorovets (VIZR)). (*http://www.agroatlas.ru/content/pests/Locusta_migratoria/Locusta_migratoria.jpg).*

The two distinct phases of locusts are solitarious and gregarious, or migratory (Lorenz, 2007). The solitary phase is the dominant phase of the species, with the gregarious phase being a physiological response to unfavourable fluctuations in the environment (e.g., lack of food). Individuals in the solitary phase are cryptic in appearance and behaviour. They avoid social contact and generally live at low densities (<3/100 m^2) (Matheson et al., 2004). They are highly camouflaged, move slowly, fly infrequently and are typically nocturnal. Locusts in this phase do relatively less harm to agricultural crops. Swarming conditions are a result of periods of high rainfall in breeding areas followed by periods of extreme drought. After the rains, large locust populations develop which then crowd together on dwindling food supplies during the subsequent drought. When food runs short they slowly cluster together and enter the gregarious phase, culminating in an aggressive swarm. Gregarious phase nymphs (*Locusta migratoria* L.) have black and yellow or orange coloration in a fixed

pattern. They have a high metabolic rate, are active (adults frequently fly during the day time) and live at high densities (100,000/100 m^2, Sword, 2000; Matheson et al., 2004).

Solitarious to gregarious phase change is triggered by olfactory, visual and mechanosensory stimulation provided by the conspecifics in the vicinity. An extremely potent stimulus involved in the phase transition is the mechanosensory stimulation of the hind legs as locusts jostle each when they are in close proximity (Roessingh et al., 1998; Simpson et al., 2001; Anstey et al., 2009). Most of a locust's integument is covered with touch sensitive hairs (trichoid sensilla) (Pflüger et al., 1981) and other mechanoreceptors (campaniform sensilla) (Hustert et al., 1981; Pflüger, 1980; Simpson et al., 2001; Rogers et al., 2003). Mechanoreceptors play an important role in locust phase transitions. Increased tactile stimulation of the hind leg (i.e., several contacts per minute over a 4-hour period), under overcrowding condition, increase the release of serotonin in the locust's thoracic ganglia. Consequently, increased levels of serotonin trigger a phase change and formation of a swarm (Simpson et al., 2001; Rogers et al., 2003). Swarms may contain up to 10^{10} individuals that cohesively travel great distances, up to 100 or more kilometres per day for an extended period of time, causing devastating crop loss (Uvarov, 1977; Topaz et al., 2008).

1.2.3.2 Swarm dynamics and individual orientation within a swarm.

Gregarious individuals tend to form swarms in several stages. First grounded, non-flying juveniles form organized bands that march along the ground. During development this marching group becomes a flying group performing short local flights and other movements which are uncoordinated with those of their neighbours. In later stages, individuals form a common spatial orientation leading to a particular shape of the group. Finally, these groups become coordinated with each other and swarm development culminates with a mass departure as adults take flight. Within a swarm individuals fly at flight speeds of 3-6 ms^{-1}

with neighbours flying in the same or different directions 0.3-9.0 m apart (Waloff, 1972; Baker et al., 1981). As the swarm propagates, it forms a rolling structure while maintaining a relatively constant shape and size (Uvarov, 1977). Although the internal motions of individuals in the swarm are not completely understood, field observations have showed that individuals within a swarm cycle through a sequence of behaviours (Fig. 1.2).

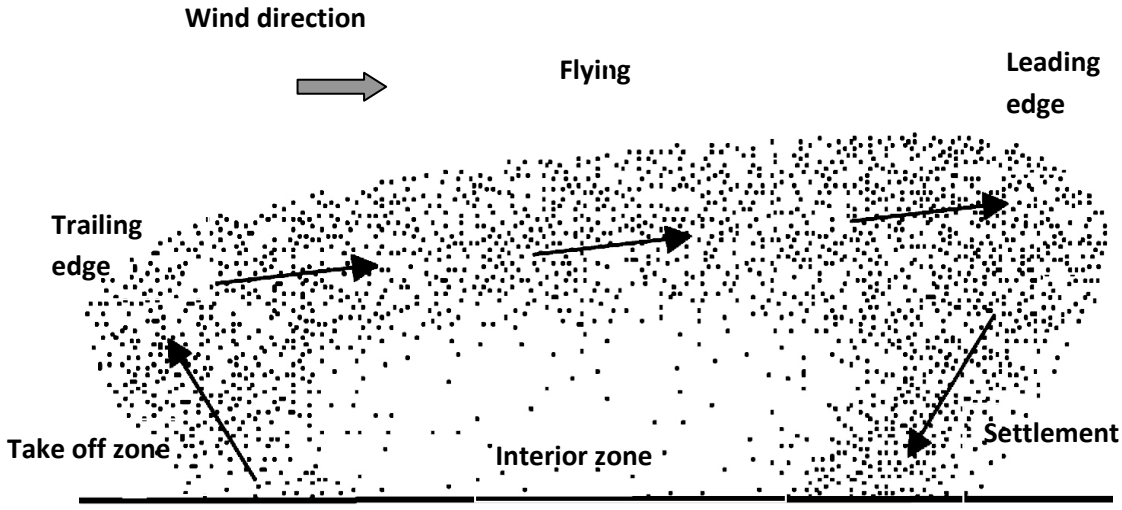


Fig. 1.2: Rolling structure of a typical swarm of the locust *Schistocerca* according to Waloff (1972) and Uvarov (1977). The zone of settlement is roughly 500m long. Rough values of the interior zone in which sporadic take-off and landing occurs, and the take off zone are respectively, 1900m and 300m long (Modified from Fig. 1 of Topaz et al., 2008).

Individuals fly towards the leading edge of the swarm usually downwind. Locusts at the front of the swarm perform a mass landing and rest, feed and oviposit on the ground until overtaken by the back or trailing edge of the swarm, at which point they take off upward and slightly upwind (i.e., in the direction between 90° to either side of the oncoming wind (Baker et al., 1984)). They then make their way to the front of the swarm until the next landing and the cycle continues (Keshet et al., 1998). According to Waloff (1972), rolling swarms are in the order of 1 km long and large swarms, with a number of rolling subunits frequently cover 10-100 km^2 or more.

Many studies have used cameras and radar techniques to study individual movement within a swarm (Waloff, 1972; Baker et al., 1981). It has been shown that locust flight is subjected to random dispersal effects and some external and internal factors such as wind speed and direction, position of the sun, the locust's age and developmental stage. According to these studies, the two major factors which have a greater influence on the orientation of day-flying locusts are orientation to the visual effects of wind and visual orientation to other individuals or to the entire swarm (Waloff et al., 1972; Uvarov, 1977; Preiss and Gewecke, 1991).

Laboratory experiments confirmed that the direction and speed of movement of individual locusts is guided by optomotor responses to pattern motion in the ventral visual field. Thus, flight orientation is related to the actual speed and direction of the wind. Optomotor responses play a major role in promoting stability in flying insects. It is a visual reflex in which the animals make a compensatory movement in response to visual motion. They turn their head or entire body in the direction of motion to keep the eyes stationary with respect to the environment and changes in air speed and flight altitude. Orientation to wind is mediated by optomotor responses to changes in the velocity of ground images over the ventral ommatidia (Kennedy, 1951). This velocity results from a vectorial addition of the locust's own flight manoeuvres and wind-induced motion. Departure from the front to back movement of images or from a preferred moderate rate of motion velocity over the retina supposedly induces compensatory responses. In these compensatory movements, locusts modulate their thrust translation to minimize the retinal velocity of ground images by reducing their movement relative to the ground. Retinal image velocity depends not only on ground speed but also on flight altitude (Preiss, 1992). Above a particular flight altitude, the maximum compensatory height, the background pattern of a locust's environment is no longer clearly detectable by the locust eye (Kennedy, 1951). Movement of resolvable images

over the eye becomes too slow to evoke responses. Accordingly, locusts are subjected to effective optomotor stimulation within the layer between the ground and maximum compensatory height. Below this height the orientation of a locust to wind is related to wind speed (Waloff, 1972).

Swarming locusts are predominantly displaced downwind (Kennedy, 1951). However the orientation of locusts in a swarm could be variable and locusts may even fly upwind at wind speeds below their flying speed (3-6 ms^{-1}) (Chapman, 1959; Waloff, 1972; Baker et al., 1984). When the wind speed approximates or exceeds flight speed, locusts turn their head and body into the wind or change their altitude (Preiss and Gewecke, 1991).

Despite the apparently random orientation of groups of individuals within the swarm, continuous cohesion of individual swarms over distances of hundreds of kilometres lasting many days has been observed without significant dispersion (Baker et al., 1984; Spork and Preiss, 1993). It is believed that optomotor responses to the relative movements of the images of surrounding individuals within a swarm are responsible for the common orientation of a group of locusts (Kennedy, 1951; Waloff, 1972). A locust changes its flight speed or direction accordingly when the translatory pattern motion in its lateral visual field changes (Spork and Preiss, 1993). Thus, individuals or groups which lose immediate contact with the swarm change their orientation and quickly head back to the swarm (Farrow, 1990). Also, swarming locusts presumably can stabilize their visual surrounding according to stimuli in their lateral visual field. This feature enables flying locusts to maintain their position relative to their immediate neighbours at least to some extent (Kennedy, 1951, Preiss, 1992). They transfer directional information and rapidly change direction away from an oncoming predator or towards a food source which has been detected by only a few members of the group. Thus, flying locusts have the ability to not only avoid predation but also effectively navigate within the swarm without constantly colliding with one another. The ability to

manoeuvre quickly and appropriately in such a dense swarm is of considerable adaptive value (Baker et al., 1981). To avoid predators and collision with conspecifics, and to navigate through complex environments, locusts must produce appropriate collision avoidance manoeuvres (Gray et al., 2001). Flying locusts rely heavily on vision for detecting obstacles in their flight path.

1.2.4 ANATOMY AND PHYSIOLOGY OF THE LOCUST VISUAL SYSTEM

1.2.4.1 Compound eye and optical reception.

An animal's ability to detect multiple sensory cues from the natural environment is crucial for its survival. More often, visual information provides the first cue about the objects that the animal encounters in its environment (Gibson, 1979). To generate adaptive behaviours for the survival, an animal's visual system should detect potentially threatening cues from contextually variable information in its visual environment (Gray, 2005).

Locusts are rapid and highly manoeuvrable flyers that can navigate through a massive swarm without colliding with one another (Uvarov, 1977). In order to perform such manoeuvring and orientation, a good sense of visual perception is important. Light is perceived by insects through a number of different receptors. Visual perception occurs via a pair of compound eyes and often three single lens eyes called ocelli. Locusts have an apposition type of compound eye, which is most common in day flying insects (Chapman, 1998) and locust vision is quite well understood. These compound eyes are constructed from many similar units called ommatidia, which provide visual inputs to the brain (Fig. 1.3). However, the arrangement and number of ommatidia differ in different parts of the eye of an insect, both within and between species. In locusts, each eye comprises 8,500 ommatidia packed in a hexagonal array (Shaw, 1978). Each ommatidium consists of a light gathering region, a corneal lens and a second lens called the crystalline cone produced by four cells

called Semper cells, and a sensory component. This sensory component consists of elongate neurons known as retinular cells which help in transforming light into electrical energy. Generally there are eight retinular cells in each ommatidium of a locust's compound eye (Wilson et al., 1978; Burrows 1996). These retinula cells extend basally as an axon into the lamina of the optic lobe through the basal lamina. The margins of each retinular cell are differentiated into closely packed microvilli which contain the visual pigment of the eye, rhodopsin. These microvilli are arranged in parallel and microvilli of each retinular cell collectively form a rhabdomere. Rhabdomeres in turn collectively form the rhabdom of the ommatidium. In a fused rhabdom, all the retinular cells within the same ommatidium have the same field of view.

In a locust, the sensory region of the ommatidium is surrounded by 16 secondary pigment cells such that each ommatidium is isolated from its neighbours (Wilson et al., 1978). The axons of retinular cells from each ommatidium project to the same region of the lamina in the optic lobe. The optic lobe is prominent anteriorly and laterally and occupies a comparatively large volume of the brain. A series of neuropil layers, the lamina, medulla, accessory medulla and lobula in the optic lobes process visual signals from the compound eye. In the lamina, the axons from each ommatidium form the cartridge. Most of the axons from the retinular cells end in the cartridge and synapse with interneurons. These interneurons receive inputs from cartridges and are known as small field cells if they receive input from one cartridge, whereas wide field cells receive inputs from several cartridges. Projections of these cells are retained in a series of columns in the medulla and the patterns of neuronal signals within the columns correspond to the image on the retina. This preservation of the image in neuronal patterns is called retinotopic mapping. There are two types of interneurons in the lobula. Wide field neurons receive input from a very large number of columns and small field neurons receive input from a small number of columns in the

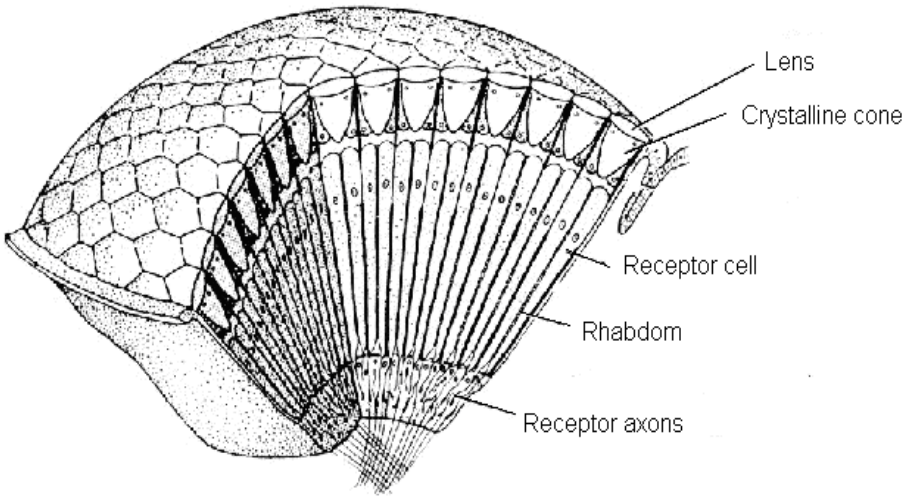


Fig.1.3**:** Schematic diagram of the apposition type of compound eye found in locusts according to Land and Nilsson, 2002 (Modified from Fig. 7.3, p 128, Land and Nilsson, 2002). In each ommatidium, the lens and crystalline cone form the light gathering apparatus. Eight microvillar photoreceptor cells/retinular cells in each ommatidium collectively form the rhabdom, the light sensing apparatus. Visual signals from the compound eye are directed to the lamina in the optic lobe via receptor axons.

medulla. Wide field neurons are known to be involved in different types of movement detection, such as looming stimuli, and provide a cue for impending collision (Simmons and Rind, 1992; Gabbiani et al., 2001; Gray et al., 2001). A well known wide field interneuron that responds to looming stimuli in locusts is the LGMD (O'Shea and Williams, 1974; Judge and Rind, 1997; Gray, 2005). The fan shaped dendritic tree of LGMD is located in the lobula and receives visual inputs from pre-synaptic visual afferents. Each right and left LGMD synapses on to a DCMD, an identified motion sensitive neuron in the lateral protocerebrum (Fig. 1.4). The synapse between these two is rapid and results in a 1:1 correspondence between presynaptic and postsynaptic spiking activity under visual stimulation (O'Shea and Williams, 1974).

Fig. 1.4: Schematic diagram of LGMD/DCMD pathway in an optic lobe and the brain of *Locusta migratoria* according to Bacon et al. (1995). Visual signals are being processed in the lamina, medulla and the lobula, respectively. The lobula giant movement detector (LGMD) interneuron receives visual inputs in the lobula and projects into the protocerebrum of the brain and synapses with the descending contralateral movement detector (DCMD) interneuron. The DCMD axon projects from the protocerebrum to the thoracic ganglia in the ventral nerve cord and excites the motor neuron and interneurons involved in initiating avoidance responses. Lam: lamina, Med: medulla, Lob: lobula, Proto: protocerebrum, Trito: tritocerebrum, Deuto: deutocerebrum, PM4: protocerebral medulla 4. This schematic diagram omits the minor branches of these interneurons.

Thus, a spike in the LGMD produces a spike in the DCMD at spike frequencies up to 400Hz (Rind, 1984). Each DCMD axon projects from the protocerebrum to the thoracic ganglia in the ventral nerve cord and in turn excites motor neurons and interneurons which are involved in jumping and in flying. This suggests that the DCMD can play a role in triggering urgent avoidance reactions in locusts (Burrows and Rowell, 1973; Simmon 1980; Santer et al., 2006).

1.2.4.2. Adaptations in the visual system involved in collision avoidance in flying locusts

Effective visually guided collision avoidance behaviours are crucial for the survival of locusts flying in a swarm. A locust's visual system has been successfully adapted to extract salient sensory cues related to looming stimuli such as conspecifics or predators (Gray, 2005; Guest and Gray, 2006). Findings from recent research have shown that the LGMD/DCMD pathway in the locust's visual system is sensitive to approaches of individual objects within a complex visual scene and these recent studies also revealed that the LGMD/DCMD system is able to respond to approaches of multiple objects approaching from different trajectories and objects approaching on a complex trajectory (Gray, 2005; Guest and Gray, 2006). It is believed that habituation in the LGMD/DCMD pathway occurs at the afferent synapses onto the LGMD (Matheson et al., 2004; Gray, 2005). Though frequent stimulation can induce habituation of looming responses, the DCMD can maintain its responses to looming stimuli approaching at a brief inter-stimulus interval e.g., 34 s. Despite that, a habituated DCMD is also able to respond to the same object approaching even at 4 s inter-stimulus interval, along a new trajectory and a larger object approaching along the same trajectory (Gray, 2005). This is because objects approaching from a new trajectory would stimulate a different, non-habituated, array of ommatidia and thus the LGMD receives inputs through different series of afferents. Also, edges of relatively larger looming objects approaching along the same trajectory would expand beyond the subtense angle of the original, smaller object and stimulate non-habituated local input elements to the LGMD. Thus, the LGMD/DCMD pathway allows locusts within a dense swarm to remain sensitive to approaches of individual objects, including conspecifics and flying predators, approaching frequently from many directions (Gray, 2005).

A locust flying in a dense swarm may encounter a complex combination of translating, receding and looming stimuli due to the self motion and object motion in the

environment. Thus, a flying locust would be subject to a combination of whole-field and small-field motion. In such situations, a locust's ability to remain sensitive to looming stimuli is very important. Whole-field movement when flying in a swarm would saturate and fatigue excitatory inputs onto the LGMD and eventually eliminate the system's ability to respond to the small-field looming stimuli. However, it is believed that inhibitory inputs to the excitatory afferents by the lateral inhibition network, reduce input to the LGMD and prevent global habituation during large-field stimulation (Rowell et al., 1977; Gray, 2005). Accordingly, localized habituation of pre-synaptic inputs to the LGMD increases the locust's ability to respond to small-field looming stimuli when flying in a dense swarm. Consequently, flying locusts have evolved a high level of sensitivity to looming objects, probably as an adaptation to avoid collision.

The locust DCMD has a wide receptive field and high sensitivity to looming stimuli approaching along a broad region of the visual field. This visual field extends from 30 to 150° azimuth and from -15 to 45° elevation (Rogers et al., 2010). Approaches from different trajectories would activate different ommatidial arrays and in turn stimulate different pre-synaptic input onto the LGMD (Guest and Gray, 2006). Thus, neurons are tuned to detect objects approaching from a wide range of directions. Interestingly, locusts display phase specific changes in their spatiotemporal receptive field and these changes are believed to account for behaviourally relevant visual stimuli linked with their life style changes. Gregarious locusts show less habituation to repeated stimuli along the eye equator than solitarious locusts do. Moreover, compared to the solitarious phase, gregarious locusts' DCMDs show a higher peak firing rate to looming stimuli in the centre of the receptive field (Rogers et al., 2007; Rogers et al., 2010). Thus, higher DCMD activity and relative resistance to habituation in gregarious locusts may allow for the sensitivity required to avoid colliding with other locusts flying in a swarm (Matheson et al., 2004; Rogers et al., 2010).

If the approaching object represents a real threat, such as predatory birds that capture locusts in flight, the avoidance responses should be executed in a timely manner (Fry et al., 1992; Santer et al., 2006; Fotowat and Gabbiani, 2011). A looming object appears to expand over the retina of the eye and the rate at which it expands increases as it gets closer to the locust. The LGMD responds vigorously to approaching objects with an increasing firing rate that decreases as collision becomes imminent (Rind and Simmons, 1992; Gabbiani et al., 2001). The timing of the peak firing rate varies linearly with the stimulus half size-to-approaching velocity ratio, occurring earlier relative to collision for larger size-to-speed ratios (large or slow looming objects). This linear relationship implies that the peak firing rate occurs at a fixed delay after an approaching object subtends a fixed angular threshold size between 15° and 40° over the locust's eye (Gabbiani et al., 1999, Fotowat and Gabbiani, 2011). The angular threshold is determined by the angular velocity, the rate at which the image travels over the retina, and the subtense angle. Thus, LGMD activity is not dependent on the object shape or texture but on the object size and its velocity. According to Guest and Gray (2006), DCMD peak firing rate, time and duration are affected by object size but are relatively insensitive to object shape. This feature in the LGMD/DCMD pathway in the locust's visual system enables locusts to respond relatively early to predators (Gabbiani et al., 2001; Rogers et al., 2010; Fotowat and Gabbiani, 2011).

1.2.5 LOCUST FLIGHT

Locust flight has been intensively studied for many years and the role of the nervous system in controlling and changing locust flight has attracted considerable research attention since the middle of the twentieth century. Locust flight is achieved by the movement of both pairs of wings and usually commences with a jump. Flight can be stimulated by the loss of

tarsal contact with the ground and by the stimulation of the aerodynamic organs on the head by an air current (Uvarov, 1977). The forewings are narrow, thick and stiff whereas the hindwings are broad and have a greater surface area. The hindwings generate 70% of the total lift during flight (Burrows, 1996). Each wing is controlled by 10 muscles innervated by about 80 motor neurons located in the 3 thoracic ganglia. These muscles attach either directly to the base of the wing (direct muscles) or indirectly causing the wings to move by distorting the shape of the thorax (indirect muscles). Some muscles, called accessory muscles (indirect muscles), modify the effects of the other muscles and cause twisting movements of a wing. Basic movement of a wing consists of repetitive up (elevation) and down (depression) movements with a twisting of the leading edge of the wing upwards (supination) during the upstroke and twisting downwards (pronation) during the downstroke. These elevated and depressed positions are achieved by contraction of sets of elevator muscles arranged vertically in the thorax and contraction of both vertically and horizontally arranged depressor muscles (Chapman, 1998).

During flight, the two pairs of wings move at the same frequency (~23 beats s^{-1}) but about 30° out of phase with each other. The hindwings reach their fully elevated position 7 ms before the forewings and their fully depressed position 4ms before (Wilson and Weis-Fogh, 1962). This rhythmic elevation and depression of the hind wings leads equivalent movements of the forewings and forms a relatively stereotyped pattern of muscle contraction (Robertson, 2004). In normal horizontal flight, locusts raise the abdomen and incline the body upwards by about 7°. Antennae are pointed directly forward, into the wind stream, and all the legs adopt a characteristic posture. That is, the front legs are folded tightly against the body, middle legs are extended posteriorly, and the hind legs point backward. When flying in a swarm, individuals have sufficient space between each other to prevent the direct interference between the wing movements of neighbours. However, factors such as turbulence and

looming objects can influence their flight direction, stability and manoeuvrability. When a locust encounters a looming object in its flight path, it could either fly around it, over it, under it or could land on it to prevent a head-on collision.

1.2.5.1 Degrees of freedom of a flying locust: translational and rotational degrees of freedom

A locust flying in 3D space has six degrees of freedom (Fig. 1.5) which is referred to as the freedom to translate forward/backward (thrust), up/down (lift), left/right (sideslip) or rotate about the roll, pitch and yaw axes (Taylor, 2001). Maintaining stability in flight is very important for flying insects. Instability in flight may involve rotation about any of the three major rotational axes passing through the centre of gravity of the body. Numerous sensory systems are involved in controlling the direction and stability of locust flight which deviates due to variations in external forces such as turbulence.

Integration of visual information from the compound eye and ocelli with mechanosensory feedback on the head and sensory feedback from the wings and the body permit stable flight and execution of the aerial manoeuvres (Taylor, 1981; Rowell, 1988; Preiss and Spork, 1993). Locusts maintain their stability in the roll plane using information from the compound eyes and ocelli, which mediate optomotor reactions (Goodman, 1965). Optomotor reactions of flying insects are part of correctional steering associated with compensating for an unintentional deviation from the course (Rowell, 1988). Hair sensilla, aerodynamic organs on the locust head, play a major role in controlling yaw (Camhi, 1970), whereas antennal scolopophores may be involved in controlling pitch (Haskell, 1960). According to Rowell (1988), steering is defined as a behaviour which induces rotation around one or more spatial axes, thus producing roll, yaw and pitch.

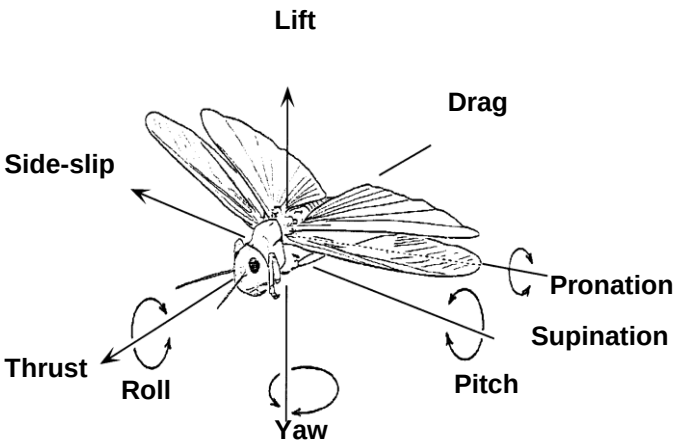


Fig. 1.5: Three rotational degrees of freedom (yaw, pitch and roll) and three translational degrees of freedom (thrust/drag, sideslip and lift) of a locust.

Locusts use several mechanisms to initiate a turn during intentional steering in thermal, auditory or collision avoidance behaviour. Asymmetric wing movements play a vital role in steering torque production in both tethered and free flying locusts (Robertson and Reye, 1992; Robertson et al., 1996; Dawson et al., 1997; Dawson et al., 2004). Thrust and lift in a flying locust are mainly produced during the downstroke (Wakeling and Ellington, 1997). To remain airborne, a locust must generate lift forces at least equal to its weight, and to move forward, the horizontal thrust vector must exceed the drag of air resisting forward motion. During a turning response, locusts increase the pronation of the inside forewing which is correlated with early pronation and consequently reduce the thrust and lift on the inside wing. However, asymmetric changes in pronation on the outside wing increase lift and thrust on the outside of the turn (Robertson and Reye, 1992, Dawson et al., 1997: Taylor, 2001).

Asymmetrical production of these two components imposes a torque on the locust and initiates a turn (Dudley, 2000). Yaw is elicited by inequality in thrust on both sides, whereas roll is generated due to inequalities in lift on either side of the centre of mass. Since, thrust

and lift are coupled during the downstroke, roll and yaw are coupled, generating a typical banking of a flying locust (Baker, 1979; Taylor, 2001; Berger and Kutsch, 2003). Roll could eventually increase the sideslip, as resultant lift forces from all wings are no longer directed vertically. Moreover, in tethered locusts, changes in the abdomen deflexion and dorsiflexion along with the asymmetrical postures of the mesothoracic and metathoracic legs in intentional steering augment the direction of the yaw and roll torques (Rowell, 1988: Santer et al., 2005). Similar postural adjustments have also been noted in bush cricket (*Tettigonia viridissima*) and cricket (*Teleogryllus oceanicus*) during acoustic avoidance responses (Schulze and Schul, 2001; Miles et al., 1992).

1.2.5.2 Looming evoked collision avoidance behaviour

Locusts respond to the objects approaching on a collision course with steering movements. Complete steering behaviour of a rigidly tethered locust involves coordinate responses of the abdomen, hind legs and wings (Robertson and Johnson, 1993a). When locusts detect a target, they flick their abdomen either directly upwards or upwards and to one side (Robert, 1989; Robertson and Reye, 1992). Abdominal movement has been considered to be an effector of steering or at least an indicator of the direction of steering. This abdominal movement affects the animal in two ways. Initially, it increases drag on the side to which it is moved and it shifts the centre of mass around which the flight forces generated by the beating wings act (Robertson and Reye, 1992).

A change in forewing asymmetry is the most consistently observed reaction to looming stimuli and suggests that a collision avoidance manoeuvre may depend more on this reaction (Robertson and Johnson, 1993a). These different wing movements underlying flight manoeuvres are controlled by changes in the relative timing and strength of contraction of particular wing muscles. In rigidly tethered and in freely flying locusts, the forewing

movements on either side are symmetrical during straight flight (Robertson and Reye, 1992; Berger and Kutsch, 2003). When the locust is exposed to the stimulus, it tends to respond to the approaching object by turning in the opposite direction. The most obvious change in the form of the wing beat associated with attempted steering is that during the downstroke the forewings become more asymmetrical whereas the hindwings remain relatively symmetrical. In addition, forewings on the inside of the turn become markedly more depressed during the wing stroke as a result of earlier stroke transition from elevation to depression for that wing (Robertson and Reye, 1992; Dawson et al., 1997; Berger and Kutsch, 2003). On the outside of the turn, the forewings remain more elevated and separated from the hind wing.

Locusts often descend to the ground from their roosts on trees by gliding flight with horizontally stretched tegmina and incompletely extended hind wings (Neville, 1965). In free flight, gliding behaviour causes the locust to dive. According to Thomas and Taylor (2001), gliding posture is aerodynamically stable as the centre of lift is above the centre of gravity. However, looming elicited gliding whilst flying is thought to be triggered when an approaching object is detected late during the loom. It is interpreted as an emergency response displayed by the flying locust to evade fast aerial predators, when other steering manoeuvres have failed (Robertson and Johnson, 1993a; Gray et al., 2001; Santer et al., 2005). In this posture, the hind wings stop moving during the downstroke of the wings (20° down: from their vertical axis) and at the same time the fore wings stop moving when they are at the top of the upstroke. In this way, the wings are elevated symmetrically and held in a swept back dihedral position above the body (Santer et al., 2005). Dihedral wing position during gliding can overcome the involuntary roll movements that would affect the stability of the gliding locust (Thomas and Taylor, 2001). Locusts usually can glide from a few to 300 ms until flapping flight is resumed (Baker and Cooter, 1979; Burrows, 1996; Santer et al., 2005). In a tethered locust, flight after a glide is more often followed by an increased wing

beat frequency. In free flight, this wing beat alteration may contribute to regaining flight stability or increasing flight speed (Santer et al., 2005). Similar flight posing behaviour has also been noted in tethered locusts in response to ultrasonic sound pulses (Dawson et al., 2004).

1.2.6 OBJECTIVES

According to previous studies, locusts avoid collision with looming objects by triggering different types of avoidance strategies along different trajectories. However, it is presently unknown how a pair of locusts responds or interacts with each other in the presence of a looming object.

I hypothesized that collision avoidance behaviour of a locust flying in a group is affected by the presence as well as relative position of a conspecific in its visual field. Based on the hypothesis, two main objectives of the present study were to determine: 1) Is collision avoidance response influenced by the presence of a conspecific? and, 2) Is collision avoidance influenced by the location of a conspecific flying within a group? In this context, the first experiment in the current study was designed to determine the effect of the presence of a second locust on the first locust's collision avoidance behaviour. Observations of locusts' responses in Experiment 1 have led to the idea that locusts use visual cues from the looming object as well as from conspecifics in the vicinity to generate appropriate avoidance behaviour. Accordingly, my second experiment was designed to determine whether different relative positions of a conspecific affect the first locust's collision avoidance behaviour. For the two experiments outlined in this thesis, I used a similar recording procedure in each of two different experimental setups.

The behavioural study described here is a new approach and the findings of these two experiments are expected to provide information about behavioural interactions between two

locusts and the way that the presence and position of a locust in the swarm affects collision avoidance behaviour. Results would further allow us to investigate neural mechanisms underlying the induction of different avoidance strategies and coordinated and cohesive movement between locusts under a dynamic visual condition.

2. MATERIALS AND METHODS

2.1 ANIMALS

Migratory locusts, *Locusta migratoria* L., were reared under crowded colony conditions (25-28 $^{\circ}$C, 12 h: 12h light: dark) in the Department of Biology at the University of Saskatchewan. They were fed with wheat seedlings and an artificial diet: wheat bran and locusts ≥ 3 weeks past the imaginal molt were selected for the experiments. All the experiments were carried out at room temperature (25 $^{\circ}$C). Only apparently healthy locusts with intact wings and uniform body lengths were chosen for the experiments.

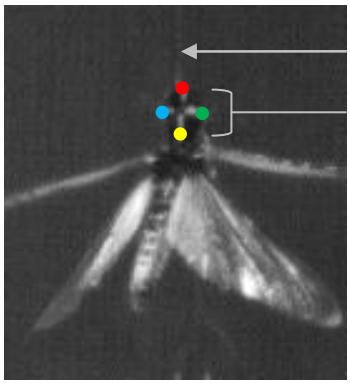
2.2 METHODS

Since rigid tethering introduces artifacts as the locust is unable to evoke complete steering behaviours, intact locusts were loosely tethered via a length of fishing line connected to a metal ring containing two vertical and two horizontal marks on the surface (Fig. 2.1A). The ring was attached to the dorsal pronotum of the locust using a small amount of low melting point beeswax and the free end of the fishing line was attached to the roof of the tunnel. Digitization of the x, y and z coordinates of four marks (see below) permitted calculation of the locust's orientation in 3D space. Tethered locusts were positioned at assigned coordinates in the wind tunnel (1 m height x 1 m width x 3 m length) and spaced 30 cm apart within a 3D volume of space (Fig. 2.1B). The mean flight speed of a locust in a swarm varies within 3-6 ms^{-1} (Baker et al., 1981). Therefore, the wind speed in the wind tunnel was set at 3 ms^{-1} to induce and maintain flight. The length of the tether, 45 cm, permitted unrestricted initial collision avoidance and initial freedom of movement in 3D space while preventing potential contact and tangling of individuals. Accordingly, loosely

tethered locusts consistently oriented upwind, maintaining a relatively fixed position (± 5 cm) in the horizontal plane of the air stream. To record avoidance responses accurately, sufficient illumination from above was provided using two lamps (LOWELPRO-LIGHT, Mfg, Inc, New York, USA). Prior to stimulus presentation in each trial, locusts were allowed to fly for approximately 5 min to allow them to adopt a stable flight posture within the tunnel. In stable flight, locusts beat their fore and hind wings symmetrically with raised prothoracic legs adducted to the pronotum, mesothoracic legs trailing down the sides of the thorax and the metathoracic legs flexed and placed below the abdomen. Subsequently, locusts were presented with looming stimuli at 2-5 min intervals, to avoid behavioural habituation. If over three consecutive trials a locust did not respond to the looming stimulus, it was not used for experimentation.

Flight behaviour was recorded from behind using two high speed video cameras (Motion scope, Redlake Camera, Inc. San Diego, USA) with an effective shutter speed of 1/120 s. The frame rate of the camera was set to 50 frames per second (fps). Two cameras were positioned at the same the height as the locusts and 1.5 m downwind. In that position, the left camera was angled ~20° to the right while the right camera was ~20° to the left. In this way, they were set up to provide two fields of view of the entire volume of space in which the locusts were free to manoeuvre. The relative position of the images of the locusts in these two cameras were synchronized and used to track the locusts' 3D flight position in different treatments. Each of the 11 treatments was replicated 20 times for a total of 220 video recordings. The duration of each recording was 40 s and the duration of the stimulus in each was 4 s (see below).

A)



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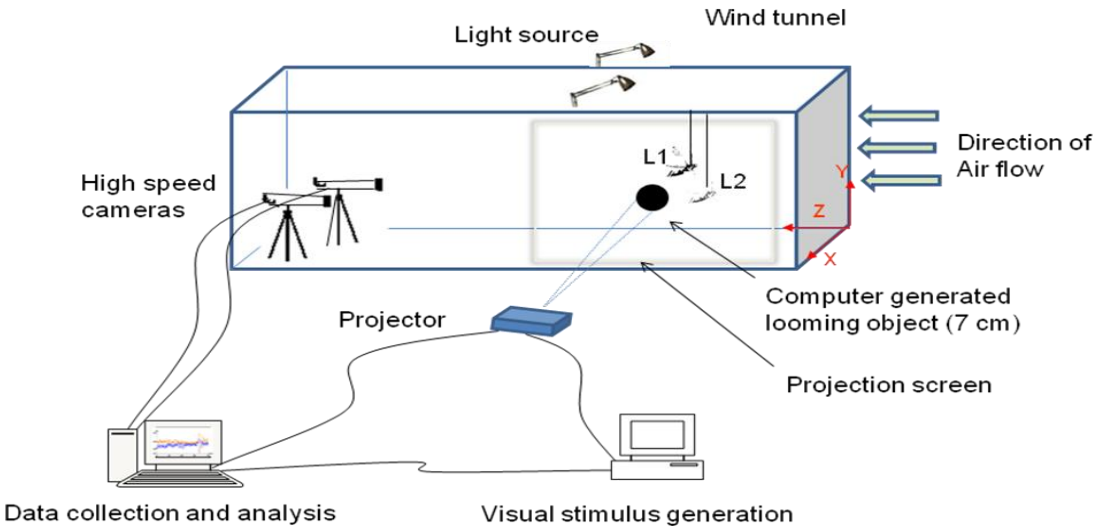


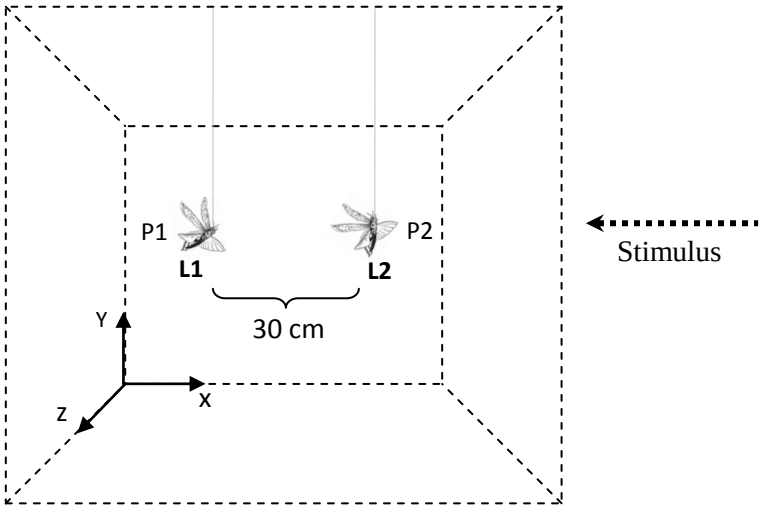
Fig 2.1: A) Image of a loosely tethered locust in the wind tunnel. Four marks i.e. two horizontal (left and right) and two vertical marks (top and bottom) on the tether were used to digitize the video frames and x, y, z coordinates and thus calculate the locust's orientation in 3D space. B) Schematic diagram of the wind tunnel and experimental setup for behavioural recording. A computer-generated disc was presented on a rear projection screen. The disc expanded along a trajectory perpendicular to the longitudinal axis of two tethered locusts positioned 30 cm apart in the wind tunnel. Airflow was produced from a fan fixed at the front of the tunnel and arrows indicate the direction of air flow. Three red arrows represent the x, y and z axes of the wind tunnel. Images recorded by two cameras at 50 fps were synchronized, saved and digitized for offline motion analysis.

2.2.1 Visual stimulus

In 11 treatments across both experiments, locusts were exposed to the same visual stimulus; a computer-generated 7 cm diameter dark disk (7 cd m^{-2}) against a white background (45.5 cd m^{-2}) with a contrast ratio of 0.73, approaching at 3 ms^{-1}. Stimuli were created using Vision Egg visual stimulus generation software (Straw, 2008) on a python programming platform. Discs were presented as 512 x 512 pixel portable network graphics (png) files. The stimulus was scaled in real time at 340 fps and projected onto a rear-projection screen (96 cm x 63 cm) placed against the right side of the wind tunnel using a Sony VPL-PX11 data projector (Fig. 2.1). Discs were presented at 0° elevation and 90° azimuth. In this orientation 0°, $+90^{\circ}$ and -90° elevation was directly at the locust eye equator, above and below the locust head, respectively. Whereas, 0° azimuth was directly in front of the locust head and 180° was directly behind. In this way, looming stimuli expanded along a trajectory perpendicular to the locust's longitudinal body axis. Thus, the centre of the looming object loomed directly towards the centre of the right eye of the locusts. Projection of the looming stimulus was triggered manually, approximately 30 s after the locust maintained stable flight. At its final position, 60 cm and 30 cm from the initial position of L1 and L2, respectively, the disk subtended 6.5° and 13° of each locust's visual field of view.

2.2.2 Set up for Experiment 1

The first experiment was designed to determine if the presence of a second locust affected collision avoidance behaviour of a locust in the vicinity. For this experiment, I used 40 adult male locusts. This experiment was divided into five treatments of which the first two served as controls (Fig. 2.2). In the controls, locusts were placed alone in two positions: P1 and P2, at two separate times. In the 3rd and 4th treatments, locusts were placed at their assigned positions separately with a dead locust adjacent to them. A dead locust with folded legs and outstretched fore and hindwings at 0° elevation angle, was oriented upwind in the same horizontal plane as the live locust. The rationale for use of a dead locust was to test whether the presence of a conspecific, irrespective of collision avoidance behaviour, would influence the first locust's response. As the final treatment, both live individuals were placed at their initial positions simultaneously.



Treatments

1. L1 at P1 } Controls
2. L2 at P2
3. L1 at P1 DL at P2
4. L2 at P2 DL at P1
5. L1 at P1 L2 at P2

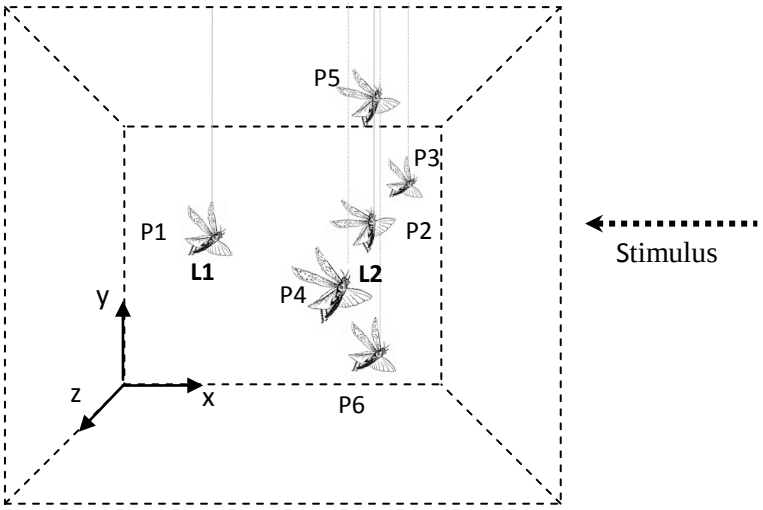
Fig. 2.2: Cross section across the wind tunnel and set up for Experiment 1. Locusts were loosely tethered and positioned at their assigned positions in the wind tunnel. The distance between two locusts within the x-plane is 30 cm. Not drawn to scale (tunnel dimensions, 1 m height x 1 m width x 3 m length). L1: locust 1, L2: locust 2, DL: dead locust, P1: position 1, P2: position 2.

2.2.3 Set up for Experiment 2

The purpose of the second experiment was to determine whether collision avoidance is dependent on the relative position of a conspecific in the vicinity. To address this question I used a different group of 40 adult male locusts. Experiment 2 was divided into six treatments. In the treatments, L1 alone at P1 and two locusts simultaneously at their assigned positions (P1 and P2) were the controls. The other four treatments involved changing the position of L2 at four different positions: P3, P4, P5 and P6 while keeping L1 at P1 (Fig. 2.3).

2.3 DATA COLLECTION

Images recorded by the cameras were saved as an .AVI file and viewed using MiDAS 2.2 (Xcitex Inc, Cambridge, Massachusetts, USA) acquisition software. Video sequences were then analyzed frame by frame using WINanalyze (Mikromak, Berlin, Germany), 3D motion analysis software. Digital analysis was performed in order to track the locust's orientation during each treatment. After 3D calibration, flight position data were converted into 3D coordinates (x, y and z) for each frame to determine how the position of each locust changed during an approach (Fig. 2.4). These coordinate values, over time, were then viewed using Dataview 6.3.2 (St Andrews University, Scotland, 1999) analysis software (Fig. 2.4). Subsequently, the numerical values from Dataview were used to determine five measurements in two experiments separately: 1) types of initial avoidance responses exhibited by each locust; 2) the direction of the initial avoidance response and extent of deviation along three translational and three rotational degrees of freedom; 3) timing of the initial avoidance response relative to projected collision; 4) duration of the initial avoidance response (i.e., time from the start to peak of the initial avoidance response);



Treatments

1. L1 at P1
2. L1 at P1 L2 at P2

} Controls

3. L1 at P1 L2 at P3
4. L1 at P1 L2 at P4
5. L1 at P1 L2 at P5
6. L1 at P1 L2 at P6

Fig. 2.3: Cross section across the wind tunnel and Set up for Experiment 2. Locusts were loosely tethered and positioned at their assigned positions in the wind tunnel. L2 was placed at four different positions in Treatments 3, 4, 5 and 6. Position numbers correspond to the treatment order and relatively smaller and larger locust images at P3 and P4 represent that in Treatments 3 and 4, L2 was in front and behind L1. In the second treatment the distance between two locusts within the x-plane was 30 cm. In Treatments 3 and 4, L2 was 20 cm ahead and behind P2 in the z-plane. In Treatments 5 and 6, L2 was 20 cm above and below P2 in the y-plane. Not drawn to scale (tunnel dimensions, 1 m height x 1 m width x 3 m length). L1: Locust 1, L2: Locust 2, P1: position 1, P2: position 2, P3: front, P4: back, P5: up, P6: down.

and 5) direction and distance between each locust at the time of the peak of the initial response.

After carefully studying each video recording, locust behaviour was categorized into the most prevalent types, such as active steering, gliding and non-directional startle response. The behaviour was categorized as active steering when locusts steered away from the looming object and flew in the opposite direction of the oncoming stimulus. Gliding was defined as cessation of the wing beat upon symmetrical elevation of the fore and hind wings and holding of the wings in an elevated position above the locust's back. Non-directional startle responses were defined as interruption of the wing beat, folding all four wings backward and extending the legs. The trials in which a particular type of response was viewed were counted and the numbers were used to determine the frequency of occurrence of each response separately.

For measurements of the three translational degrees of freedom, forward, upward and right translation were designated as positive values, relative to each locust's initial position whereas backward, downward and left movements were designated as negative values. Additionally, for measurements of the three rotational degrees of freedom, for both yaw and roll, a positive angle value indicated rotation to the right (clockwise) and upward rotation for pitch. The changes in three rotational degrees of freedom in successive frames were determined relative to a set of virtual axes centered on the locust body at time 0 s. In Experiment 1, changes in three rotational degrees of freedom were assessed using the differences between x, y and z coordinates of all four marks: top, bottom, left and right, on the tether. However, changes in the three translational degrees of freedom in Experiments 1

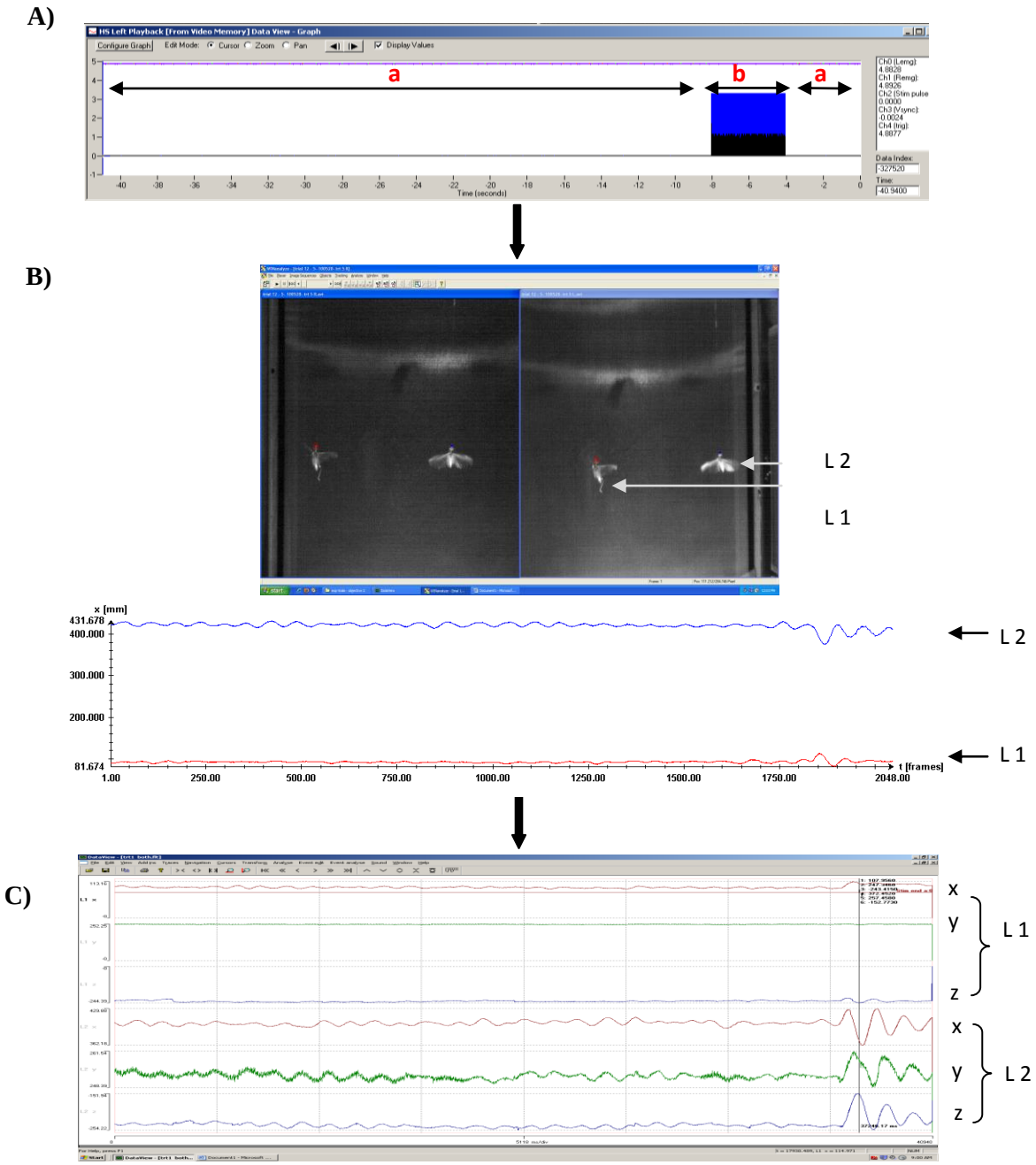


Fig. 2.4: Analysis of flight position data. A) MiDAS screen shot illustrating the duration of the recording (i.e., a + b + a) and the looming stimulus (b). Note that the total duration of the recording was 40 s and the stimulus duration was 4 s. B) Top panel: Screen shot taken from Winanalyze, showing the synchronized and digitized two video frames taken by left and right high speed cameras. Following the digitization, L1 and L2 flight position data were converted into 3D coordinates. Bottom panel: illustration showing how the L1 and L2 flight positions deviate along the x-axis over 40 s recording period. Greater deviation along x axis indicates the steering of L1 and L2 to the left. C) Digital flight position data of L1 and L2 in Dataview. x, y and z coordinates of both locusts have been displayed in one time scale. Thus, it allows determination of how the flight position changes along three axes at the same time and timing of the responses during recording. L1; locust 1, L2; locust 2.

and 2 were assessed by taking the difference between x, y and z coordinates of the top mark at the onset and the peak of the initial avoidance response.

For the 3rd measurement, timing of the initial avoidance response was considered as the time of the video frame in which a first indication of an avoidance response, such as abdomen deflection, abdomen dorsiflection, hind leg extension and flight cessation, was visible. For the 4th measurement, the time spent for the initial response was determined by taking the time difference between the onset and the peak of the initial avoidance response, the frame in which highest magnitude of the initial response was visible prior to the projected collision time.

Direction and distance between each locust at the time of the peak of the initial response was determined in each video recording. In addition, to determine whether the two locusts responded to the looming object as a pair or individually, the number of trials in which both locusts flew in the same direction (i.e., either to the left or right) at the onset of the response was counted. These trials along with the rest of the trials were used to calculate the probability of the grouping behaviour.

2.4 DATA ANALYSIS

To determine the relative response in each treatment, data from measurements 2, 3 and 4: extent of deviation along six degrees of freedom, time of the initial response and duration of the initial response, respectively, were first normalized to those of the control treatments for each animal. In the normalization, relative values in control treatments were considered as 1 for measurement 2, and 0 for measurements 3 and 4. For the 2nd measurement, normalization was carried out by dividing treatment values by the control value (1). For the 3rd and 4th measurements, treatment values were subtracted from the control (0). SigmaStat 3.5 was used to compare both normalized and non-normalized deviation and

timing data between treatments as well as with those of the control and plotted using SigmaPlot 10.0 (Systat Software Inc., Richmond, CA, USA). Data were tested for normality and equal variance. Parametric data were compared with a one way ANOVA whereas non-parametric data were compared with a Kruskal-Wallis one way ANOVA on Ranks. Significant differences between treatments were assessed using post-hoc pair-wise multiple comparison procedures (Tukey Test or Dunn's test). Treatments were declared significantly different at $P < 0.05$.

3. RESULTS

3.1 EXPERIMENT 1:

3.1.1 Types of initial avoidance responses

Locusts responded to the looming disc with a variety of responses, including active steering, gliding and non-directional startle responses. Active steering involved coordinated responses of the abdomen, hind legs and wings. As the first sign of active steering (22 of 38), near the end of the stimulus approach, locusts flicked their abdomen directly upwards and/or upwards and to one side (to left or right). Then, locusts oriented towards the approaching stimulus and suddenly turned away from (to the left of) the looming object by extending the left hind leg to the same side as the left hind wing. Finally, active steering resulted in flying away from the stimulus (Fig. 3.1A).

Another group of locusts (11 of 38) demonstrated gliding behaviour. In this context, near the end of stimulus approach, wing beating ceased and the wings were held in a gliding posture, elevated above the locust's back. Gliding lasted for 100-200 ms (Fig. 3.1B). As in normal steady flight, both fore and hind wings were symmetrical in the elevated position. Immediately after gliding, six locusts resumed the wing beat cycle and the remaining five ceased flight by folding their fore and hind wings back over the abdomen.

Five locusts exhibited two types of startle responses in addition to active steering and gliding behaviour. The first type involved interruption of the wing beat, folding of all four wings backward and extending the legs out. In tethered flight, this type of startle response ended with a sudden movement towards the forward in the z-plane (Fig. 3.1C). As the second type, two locusts responded to the stimulus by deflecting the abdomen and hind

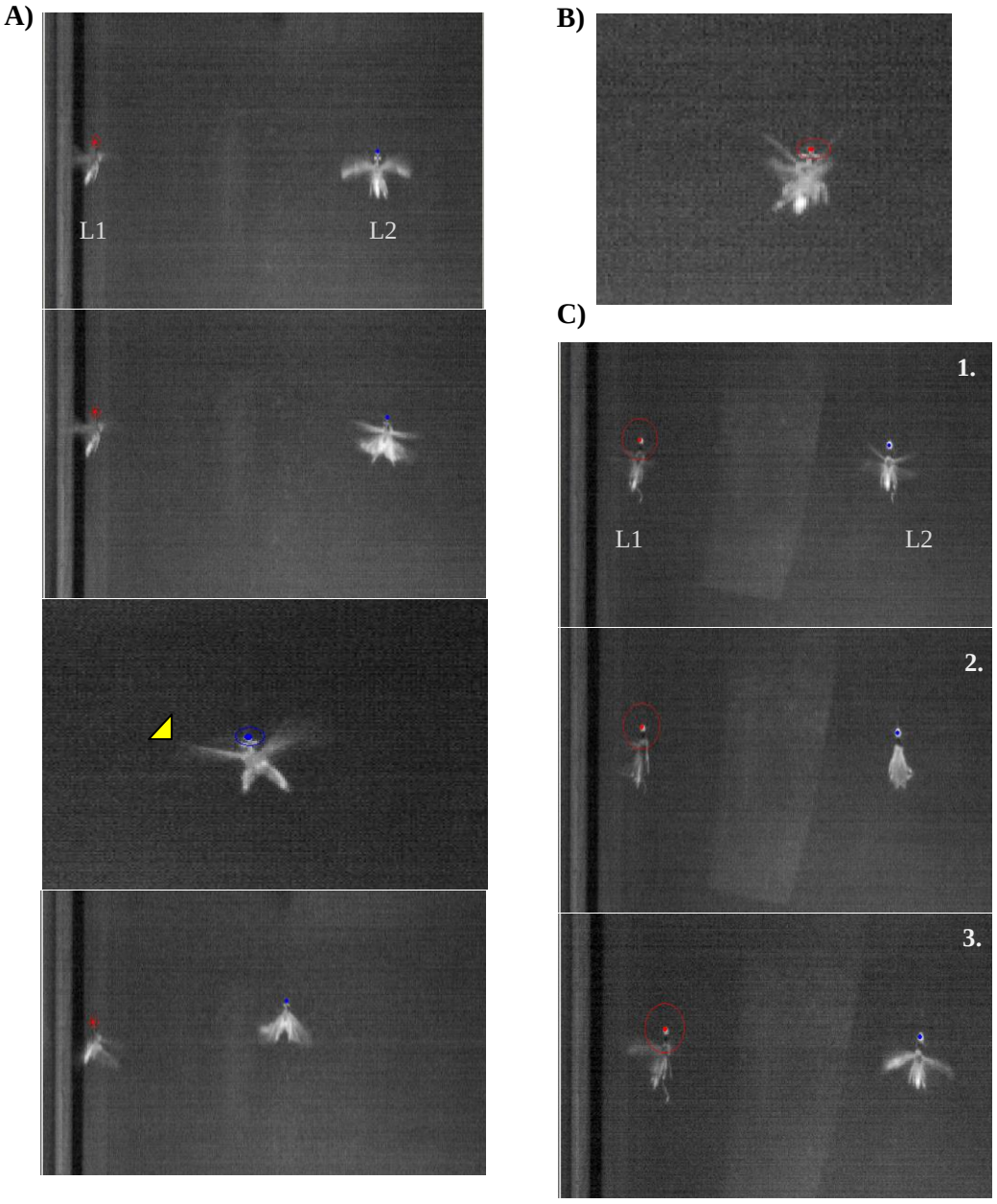


Fig. 3.1: Single frames demonstrating three types of avoidance responses evoked by a looming stimulus from the right. A) Four video frames from Treatment 5, illustrating the steps involved in active steering of L2 as viewed from behind, when L1 was alive. 1. Steady flight. 2. Turning of the whole body towards the direction of the looming stimulus 3. Active steering with more depressed left fore wing on the inside of the turn. Position of the left forewing is indicated with an arrow head. 4. Resulting movement from active steering i.e. to the opposite direction (left) of the looming stimulus. B) Gliding posture of a locust viewed from behind: fore and hind wings are maintained in an elevated position. C) Three video frames from Treatment 5, illustrating the steps involved in Type 1 non-directional startle response of L2. 1. Steady flight of L2 as viewed from behind. 2. Folding of all four wings and movement to the forward direction. 3. Resumption of flight after 180 ms. Frame A-3 and B are on different spatial scales relative to the other frames as they are magnified to highlight the responses described in particular. L1: Locust 1; L2: Locust 2.

legs and rapidly flying towards 315° 45° (a 90° sector at the front of the locust in the wind tunnel) azimuth range in the horizontal plane. In this study, both types of startle responses were considered as the non-directional startle response.

Figures 3.2A and B illustrate the percentage occurrence of three responses of Locust 1 (L1) and Locust 2 (L2), respectively. L1 and L2 exhibited all three types of behaviours in three treatments. However, responses of L1 in these three treatments were not consistent (Fig. 3.2A). Gliding behaviour was most prominent (53%, n = 10) when L1 was alone in control/Treatment 1 and was exhibited with the least (21%, n = 4) and second highest frequencies (32%, n = 6) when paired with a live and dead locust (DL) in Treatments 5 and 3, respectively. However, when paired with DL (47%, n = 9) and a live locust (42%, n = 8), L1 responded to the looming stimulus most frequently by steering away from it. Among three types of responses, non-directional startle response had the lowest and second highest percentage in Treatments 1 (5%, n = 1) and 3 (21%, n = 4) whereas, among three treatments, performance of the startle response was highest in Treatment 5 (37%, n = 7).

As illustrated in Figure 3.2B, the percentage occurrence of the three types of responses in L2, followed the same trend in three treatments. Even though L2 was alone in control/ Treatment 2 (74%, n = 14) and paired with a dead (58%, n = 11) and a live locust (79%, n = 15) in Treatment 4 and 5, the majority of animals avoided the stimulus by actively steering away from it. The highest percentage occurred when L2 was paired with a live locust in Treatment 5. Gliding behaviour was the second most frequently occurring behaviour in three treatments and it was highest when L2 was coupled with a DL in Treatment 4 (32%, n = 6). In all three treatments, startle responses occurred least often (11%, 11%, 5%, n = 2, 2, 1) indicating the least possible type of escape strategy taken by L2 throughout the treatments.

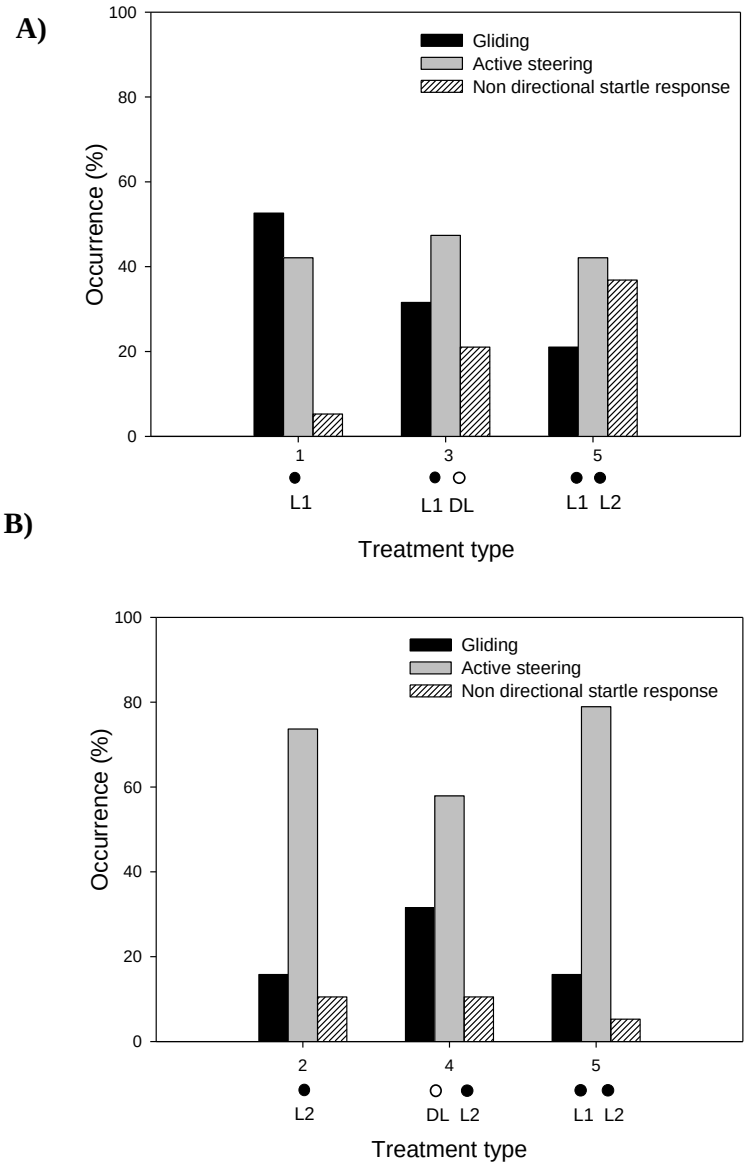


Fig. 3.2: Percentage of occurrence of three responses; gliding, active steering and non-directional startle response of (A) L1 (*n*=19) and (B) L2 (*n*=19) in response to looming stimuli during Experiment 1. Each column is the percentage of trials in which particular type of behaviour was evident. L1: Locust 1; L2: Locust 2; DL: Dead locust.

3.1.2 Direction of initial avoidance responses and extent of deviation along six degrees of freedom.

The distribution of L1 and L2 flight paths in different treatments was determined using flight position coordinates in successive video frames between the onset and peak of the initial avoidance response (Fig. 3.3). As illustrated in (A) and (B), L1 flight tracks in Treatments 1, 3 and 5 were confined to a smaller range along the x-axis (± 100 mm). This implies a smaller lateral deviation of L1 during collision avoidance. Conversely, L2 flight tracks were extended in a wide range along the x- axis (± 250 mm), implying a greater movement to the left and right directions in response to looming stimuli.

Compared to the lateral deviation, L1 and L2 movement along the z-axis followed a similar trend. Both locusts exhibited a greater forward movement (+ 125-150 mm) and a relatively smaller backward movement (- 50 mm) along the z-axis in different treatments. Overall, L1 showed greater forward movements when it was alone in Treatment 1. In terms of L2, greater forward movements occurred when it was alone in Treatment 2 and coupled with L1 in Treatment 5. However, compared to the deviation along the x and z axes, upward and downward movements of both locusts along the y-axis were relatively small. Regardless of the presence or absence of a dead or live locust, L1 demonstrated less movement along the y-axis and therefore less deviation in elevation during different trajectories. However, L2 movement along the y-axis deviated more than the L1 and higher elevations were demonstrated when L2 was alone in Treatment 2 and paired with a L1 in Treatment 5.

3.1.2.1 Three translational degrees of freedom

Deviation of three translational degrees of freedom (i.e., sideslip, lift and thrust) of both locusts was plotted against the three treatment types (Fig. 3.4). The behaviour in each treatment was normalized to that of the control to obtain the relative response and compared

with that of the related control treatments in which L1 and L2 were alone (Treatments 1 and 2). Comparisons were done to determine whether collision avoidance behaviour of L1 and L2 was affected by the presence of a conspecific.

A Kruskal-Wallis ANOVA on ranks showed that there was a significant effect of the treatments ($P < 0.05$). Figure 3.4A shows how the sideslip translation of L1 and L2 change over the 40 s recording period in Treatment 5. As shown in Figure 3.4B, three translational degrees of freedom of L1 demonstrated an inconsistent pattern in overall trend throughout the experiment. Compared to the control, sideslip translation increased whereas lift decreased. Also, the presence of a dead and a live locust evoked a greater fluctuation in L1 thrust translation (Fig. 3.4B). Compared to the control, L1 total sideslip movement, which is considered as a combination of both left and right movement (Dunn's Method, $n = 19$, $H_2 = 16.999$, $P < 0.001$) and left movement (Fig. 3.4B) was significantly higher (Dunn's Method, $n = 19$, $H_2 = 12.150$, $P = 0.002$) with greater variability when paired with a dead and a live locust in Treatments 3 and 5.

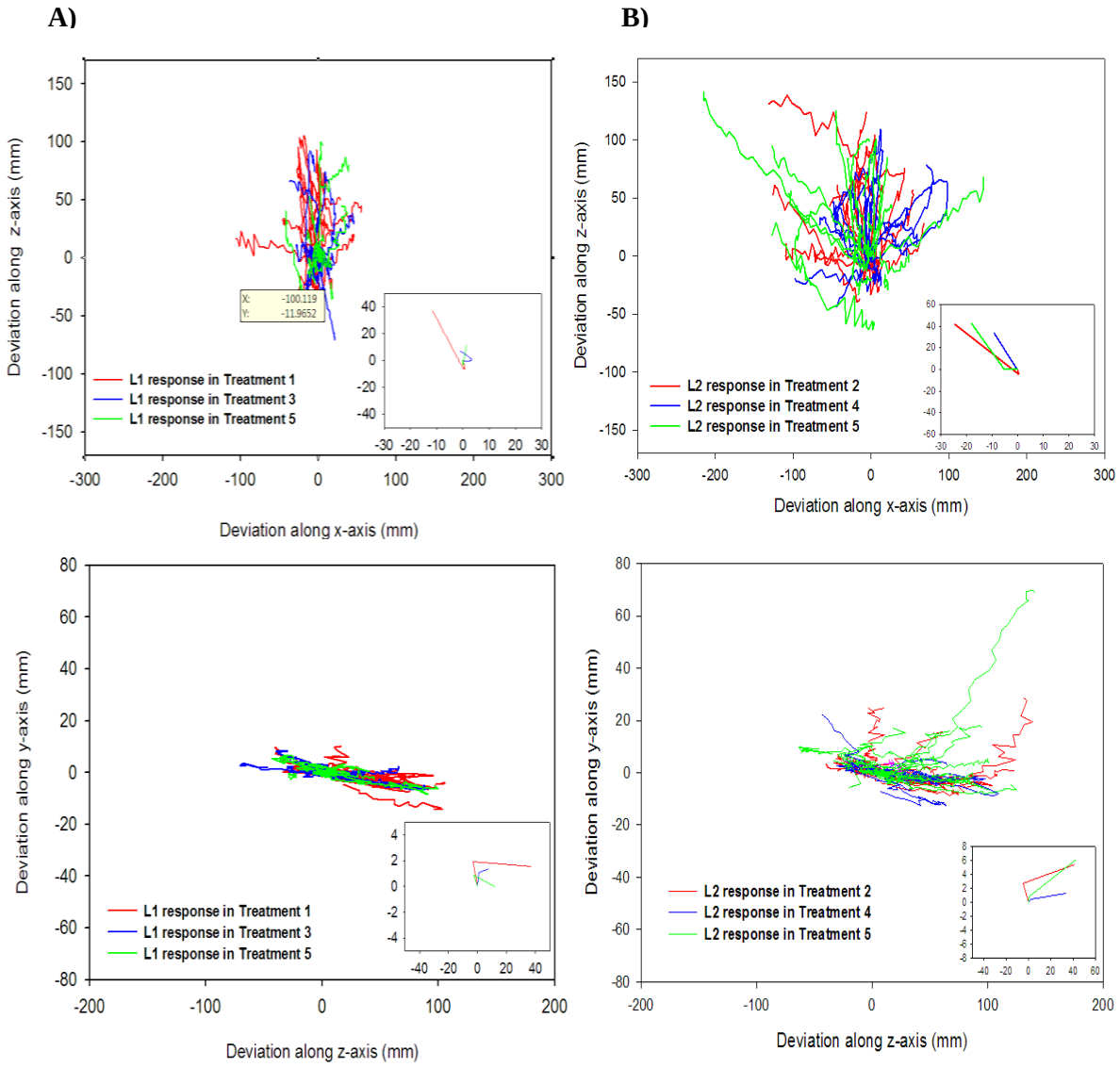


Fig. 3.3: Summary of the flight paths of L1 (n=19) and L2 (n=19), in response to the laterally looming stimuli. A) Top panel: L1 movement along the x-axis against movement along the z axis as viewed from above the wind tunnel. Bottom panel: L1 movement along the z-axis against movement along the y-axis as viewed from the right side of the wind tunnel. B) Top panel: L2 movement along the x- and z-axis as if viewed from above the wind tunnel. Bottom panel: L2 movement along the z- and y-axis as if viewed from the right side of the wind tunnel. The inset in each plot clearly demonstrates the direction and average deviation of motion of initial collision avoidance response of L1 and L2 in each treatment. Greater average deviations in the motions are along the x (- 30) and z (+ 60) axes. Compared to x and z, average movements along y-axis is low (+ 8). Relative to the initial position at the start of the recording (zero), positive and negative deviations along the x-axis respectively, represent the rightward and leftward movements during the initial avoidance response; whereas the positive and negative deviations along the z-axis represent forward and backward movements, respectively. Upward movements are positive and downward movements are negative along the y-axis. L1: Locust 1; L2: Locust 2.

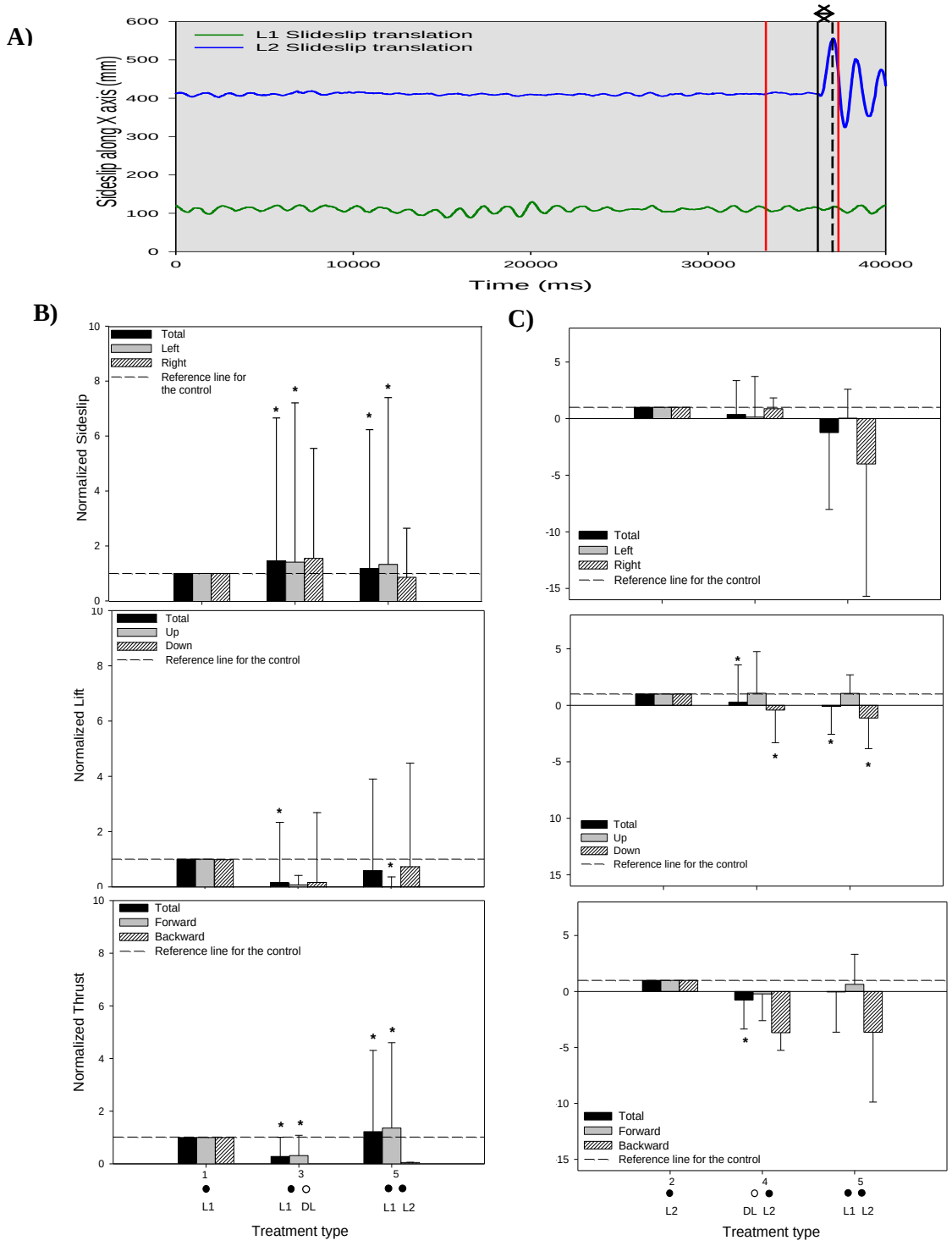


Fig. 3.4: A) Example of data from a single trial showing sideslip deviation of L1 and L2 along the x-axis over a 40 s recording period. Space between red lines indicates the duration of the looming stimulus (4s) and black solid and dashed lines represent the onset and the peak of the initial avoidance response respectively. The area marked by double arrow shows the extent of deviation and direction of the initial response of L1 and L2 in Treatment 5. Upward and downward deflections represent the movement to the right and left, respectively. B) Statistical summary of direction of initial avoidance response and normalized extent of deviation of 3 translational degrees of freedom of L1 (n=19) and C) L2 (n=19). Error bars represent the standard deviation. Comparisons were made between treatments and asterisks indicate significant differences with the control at $P < 0.05$. L1: Locust 1; L2: Locust 2; DL: Dead locust.

Also, overall lift including total, upward and downward movements in Treatments 3 and 5 was lower compared to the control. Statistically, total lift translation, which is the combination of upward and downward movement (Dunn's Method, $n = 19$, $H_2 = 10.085$, $P = 0.006$) and upward movement (Tukey Test, $n = 19$, $H_2 = 7.692$, $P = 0.013$) of L1 were significantly lower in the presence of a dead and live locust (L2), respectively (Fig. 3.4B). There were also significant differences in thrust/ drag of L1 between the treatments (Fig. 3.4B). Total thrust, which is the combination of forward and backward movements (Tukey Test, $n = 19$, $H_2 = 21.156$, $P = < 0.001$), and forward movement (Tukey Test, $n = 19$, $H_2 = 17.308$, $P < 0.001$) of L1 was significantly lower and less variable when paired with a dead locust and significantly higher and more variable when coupled with a live locust in Treatment 5. In both treatments, backward movement of L1 was less pronounced.

Although there was a decrease in the total and left movement of L2 among treatments (Fig. 3.4C), there was no statistical difference in sideslip translation. However, relative to the initial response, L2 exhibited a reverse total sideslip and right movement with greater variability when coupled with a live locust in Treatment 5. Also, compared to the control, both total (Tukey Test, $n = 19$, $H_2 = 13.855$, $P =< 0.001$) and downward movement (Tukey Test, $n = 19$, $H_2 = 9.848$, $P = 0.007$) of L2 along the y-axis was significantly affected by the presence of DL and L1 (Fig. 3.4C). L2 downward movement in Treatments 4 and 5 and the total lift in Treatment 5 was reversed relative to the initial response in the control. Compared to the control, L2 exhibited more pronounced backward movements when coupled with a dead and live locust in Treatments 4 and 5. Total movement of L2 in Treatment 4 differed significantly (Tukey Test, $n = 19$, $H_2 = 5.793$, $P = 0.04$) from the control (Fig. 3.4C). In addition, relative to the initial response in the control, total thrust including forward and backward response was reversed in Treatments 4 and 5.

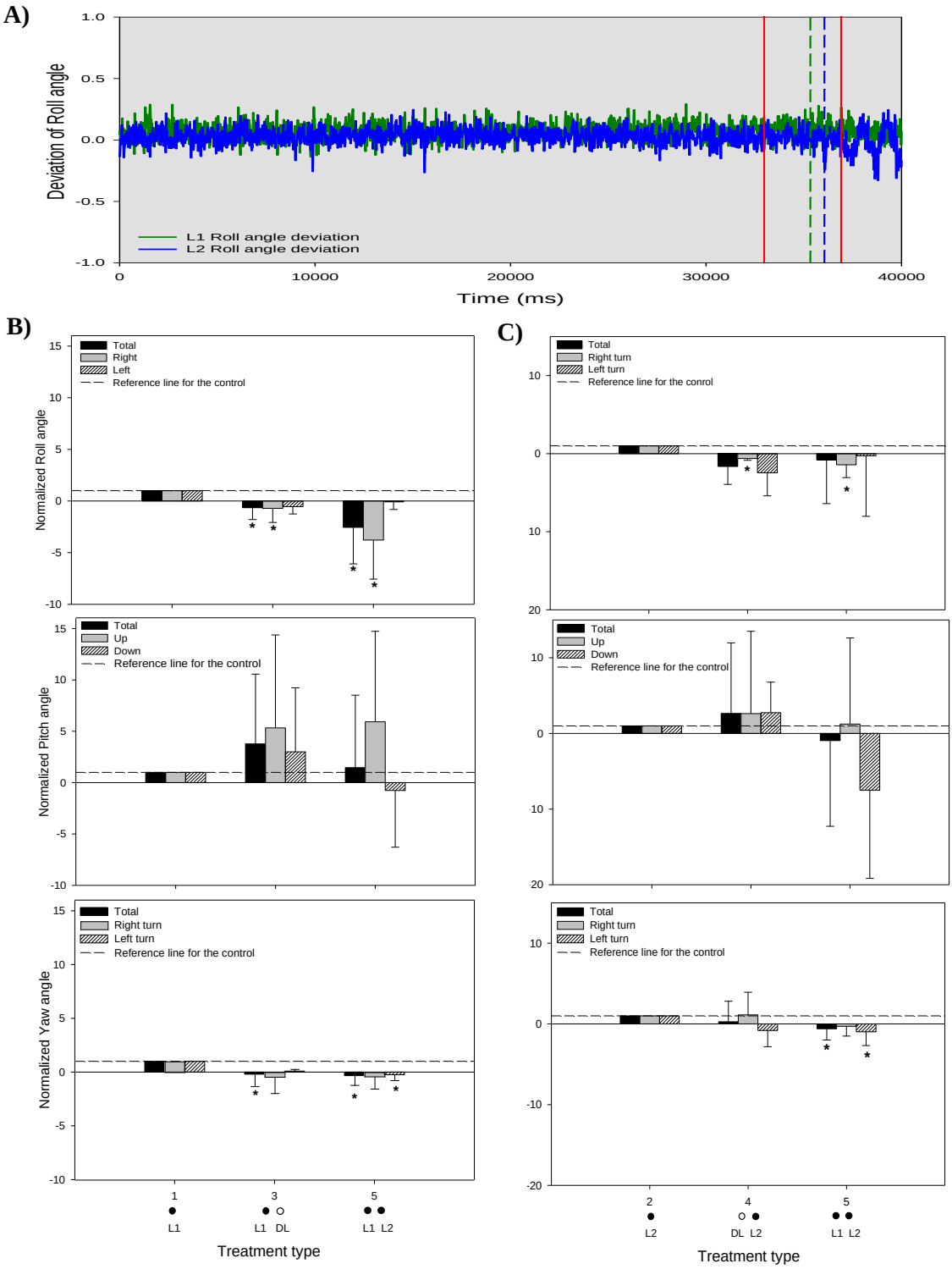


Fig. 3.5: A) Example of data from a single trial showing the roll angle deviation of L1 and L2 over a 40 s recording period. Space between red lines indicates the duration of the looming stimulus (4 s) and green and blue dashed line represents the onset of the initial avoidance response of L1 and L2, respectively. A positive value in the x-axis indicates rotation to the right and a negative value represents rotation to the left. B) Statistical summary of direction of initial avoidance response and normalized extent of deviation of three rotational degrees of freedom of L1 (n=9) and C) L2 (n=9). Error bars represent standard deviation. Comparisons were made between treatments and asterisks indicate significant differences with the control at $P < 0.05$. L1: Locust 1; L2: Locust 2; DL: Dead locust.

3.1.2.2 Three rotational degrees of freedom

Compared to the control, there were significant differences in the roll and yaw rotation of Locust 1 (L1) and Locust 2 (L2) in different treatments (Fig. 3.5). Roll and yaw angles of L1 demonstrated significant changes in response to the looming stimulus in Treatments 3 and 5 in which L1 was paired with DL and L2 (Fig. 3.5B), whereas the pitch angle did not show significant changes throughout the experiment (Fig. 3.5B). Regardless of the presence of a dead or a live conspecific in Treatments 3 and 5, overall roll angle responses of L1 were reversed relative to that of the control. Compared to that of the control, roll angles to the right differed significantly in both treatments (Tukey Test, $n = 9$, $H_2 = 13.022$, $P = 0.001$). Significant differences were also found in total roll rotation (Tukey Test, $n = 9$, $H_2 = 18.595$, $P < 0.001$). Even though there were no significant differences in the L1 pitch angle, L1 responses were relatively high with greater variability in Treatments 3 and 5. On the other hand, the yaw angle was weaker and less variable in Treatments 3 and 5. However overall, responses were reversed relative to the control. The presence of a dead locust in Treatment 3 evoked a significant difference in the total yaw rotation (Tukey Test, $n = 9$, $H_2 = 11.013$, $P = 0.004$). Additionally, there were significant differences in Treatment 5 in terms of total yaw rotation (Tukey Test, $n = 9$, $H_2 = 11.013$, $P = 0.004$) and yaw angle to the left (Tukey Test, $n = 9$, $H_2 = 7.812$, $P = 0.01$) (Fig. 3.5B).

As with L1, roll and yaw angles of L2 demonstrated significant changes in Treatments 4 and 5 in which L2 was paired with DL and L1 (Fig. 3.5C). Relative to the control, overall roll rotation was reversed and roll angle to the right was significantly affected by the presence of DL and L1 (Tukey Test, $n = 9$, $H_2 = 7.652$, $P = 0.015$). As illustrated in Figure 3.5C, the presence of a dead or a live locust did not have a significant effect on L1 pitch rotation but, relative yaw rotation of L2 decreased in Treatments 4 and 5. When L2 was paired with L1, there were differences in the yaw total (Tukey Test, $n = 9$, $H_2 = 10.67$, $P =$

0.005) and left turn (Tukey Test, $n = 9$, $H_2 = 7.812$, $P = 0.011$) (Fig. 3.5C). Compared to the control, this yaw total and left turn in Treatment 5 was significant and reversed. As illustrated in Figures 3.4 and 3.5, locusts tended to respond to a looming object more vigorously in the presence of a live locust than a dead locust, significantly altering the three translational and two of the three rotational degrees of freedom when flying in 3D space. These findings suggest that a locust's initial avoidance response is significantly affected by the presence of a conspecific in its vicinity, especially when the conspecifics alive.

3.1.3 Timing of the initial avoidance response relative to projected collision

Timing of the initial avoidance response of L1 ($n = 19$) and L2 ($n = 19$) in each treatment was compared to determine if there was an effect of the presence of a conspecific on the timing onset of the initial avoidance response. According to Figure 3.6B, the median time of L1 response onset in the control was 766 ms before the projected collision time. Also, in Treatments 3 and 5 the median time of L1 response onset was 724 and 823 ms, respectively, before projected collision time. Even though, compared to the control, L1 responded 57 ms earlier when paired with a live locust and 42 ms later when paired with a dead locust, there was no significant difference in L1 time relative to collision, either between treatments and with the control (Fig. 3.6B). L1 responded to the looming object at almost the same time in all three treatments indicating that the time of the initial avoidance response of L1 was unaffected by the presence of a conspecific in its visual field.

Conversely, L2 responded earlier when paired with DL and L1 in Treatments 4 and 5, respectively. However, when coupled with a live locust (L1 in Treatment 5), L2 responded significantly earlier compared to the control (Tukey Test, $n = 19$, $H_2 = 6.530$, $P = 0.038$) (Fig.

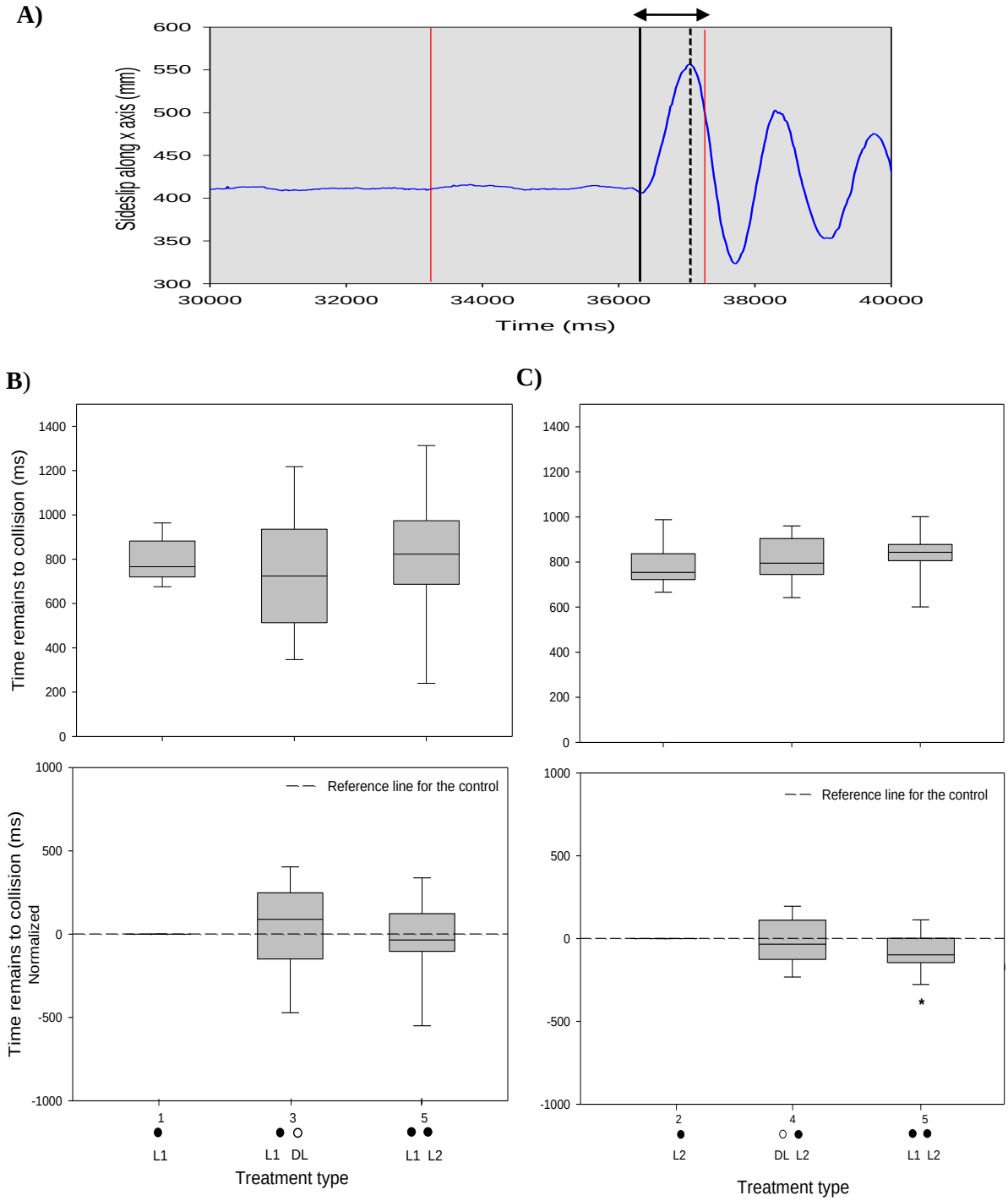


Fig. 3.6: A) Example of actual data from a single trial showing the sideslip deviation of L2 over the final 10s of the recording period. Space between red lines indicates the duration of the looming stimulus (4s) and black solid and dashed lines represent the onset and peak of the initial avoidance response of L2. Time to projected collision, which is indicated by a double arrow, was taken as the difference between onset of the response and projected collision time. B) Effect of treatment on initial avoidance response time of L1 (n=19) and C) L2 (n=19). The 1st and 2nd panel indicates the raw time values and normalized time values, respectively. Boxes show the 25th and 75th percentiles and median value and whiskers show the 5th and 95th percentile level. The asterisk indicates significant difference from the reference value (dashed line) at $P < 0.05$. L1: Locust 1; L2: Locust 2; DL: Dead locust.

3.6C). This result suggests that the presence of a live conspecific affects the timing of the initial response onset of a locust in a group.

3.1.4 Duration of the initial avoidance response

The duration of the initial avoidance response was measured to determine if it was affected by the presence of a conspecific. As illustrated in Figure 3.7B, the duration of the initial response of L1 in three treatments was similar and there were no significant differences between treatments or with the control.

Overall, compared to L1 response duration, the L2 response duration in three treatments was slightly longer (~ 50 ms), indicating a slower reaction than L1 (Fig 3.7C). However, compared to the control treatment in which L2 was alone, response duration decreased by 30 and 15 ms (difference between the median of the response duration) when L2 was coupled with a dead and live locust in Treatments 4 and 5 (Fig. 3.7C). However, these differences were not significant. Regardless of the presence of a dead or a live locust, the response durations of both locusts were the same in different treatments. These findings imply that the presence of a conspecific does not affect the response duration or reaction speed of a locust in a group whether it is nearer or further from the stimulus.

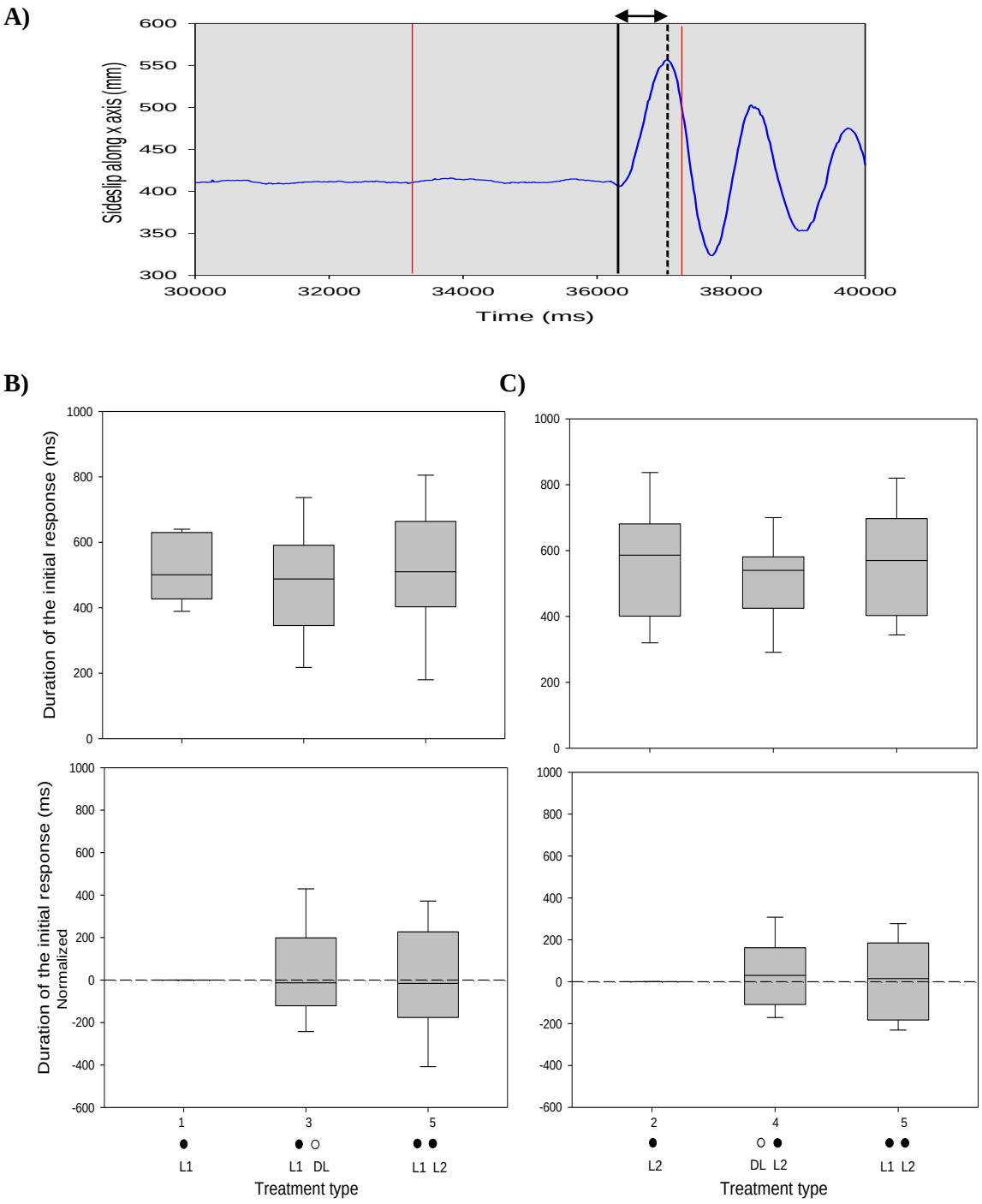


Fig. 3.7: A) Example data from a single trial showing the sideslip deviation of L2 over the final 10 s of the recording period. Space between red lines indicates the duration of the looming stimulus (4 s). Black solid and dashed line represents the onset and the peak of the initial avoidance response respectively. Thus, the double arrow represents the duration of the initial avoidance response of L2 in treatment 5. B) Effect of treatment on duration of the initial avoidance response time of L1 (n=19) and C) L2 (n=19). The 2nd and 3rd panels indicate the raw and normalized time values, respectively. Boxes show the 25th and 75th percentiles and median value and whiskers show the 5th and 95th percentile level. L1: Locust 1, L2: Locust 2; DL: Dead locust.

3.1.5 Direction and distance between each locust at the time of the peak response.

At the beginning of the Experiment 1, two locusts were kept 30 cm apart from each other (in Treatment 5). However, at the time of the initial avoidance response, the two tethered locusts were able to maintain a distance which was closer to 30 cm at the beginning. On average (n = 19), the distance between the two at the onset of the initial response was 32 cm (data not shown). The time of the peak response was measured as the time of the video frame in which the highest magnitude of the initial response was visible prior to the projected collision time. At the time of the peak avoidance response, the average distance between each locust was 30.6 cm (Fig. 3.8). Although this distance was slightly low compared to that of the onset of the response and higher than that of the set value, there was no significant difference among the distances.

To determine whether the two tethered locusts responded to the looming object as a pair or individually, the number of trials in which both locusts flew to the same direction (i.e., either to the left or right), during the initial response, was counted. Consequently, 68% of locust pairs (n = 13) steered the same direction while 32% of locust pairs (n = 6) steered in opposite directions (i.e., either towards or away from each other). Results imply that two locusts tend to respond to the looming object as a pair and demonstrate some ability to maintain the distance from a neighbour during collision avoidance.

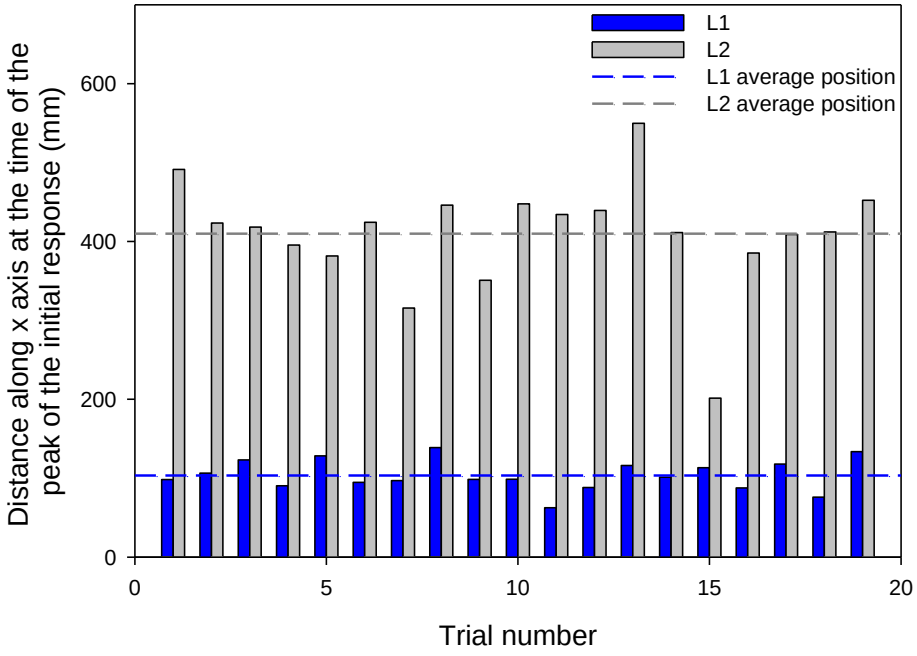


Fig. 3.8: Position of L1 (*n*=19) and L2 (*n*=19) along the x-axis at the time of the peak of the initial response at each trial in Treatment 5. Blue and grey dashed lines show the average position of L1 and L2 along the x-axis at the peak of the initial response, respectively. Thus, distance between the two dashed lines represents the average distance between the locusts at the time of the peak of the initial avoidance response. L1: Locust 1; L2: Locust 2.

3.2 EXPERIMENT 2:

3.2.1 Types of initial avoidance responses

Looming stimuli triggered three types of avoidance responses including active steering, gliding and non-directional startle responses in L1 and L2. Figures 3.9A and B illustrate the percentage occurrence of three responses in L1 and L2, respectively. L1 exhibited all three behaviours in six treatments. Nevertheless, no consistent trend among responses was observed. A non-directional startle response was the most prominent type of response in all treatments. Percentage occurrence was highest in Treatment 1 in which L1 was alone (72%, n = 13) and lowest in Treatment 4 (44%, n = 8) when L2 was behind L1 (Fig. 3.9A). In Treatments 3, 5 and 6, gliding behaviour had the second highest frequency and it was followed by active steering, which had the lowest percentage occurrence among three response types. Conversely, in Treatments 1 and 4, gliding behaviour showed the lowest percentage indicating the lowest occurrence (11% and 17%, n = 2 and n = 3). When L1 was parallel to L2 in treatment 2, gliding and active steering occurred at a similar frequency (22%, n = 4).

As illustrated in Figure 3.9B, percentage occurrence of the three responses in L2 did not follow the same trend. In Treatments 2, 3 and 4 in which L2 was parallel, in front and behind L1, respectively, the majority of animals avoided the stimulus by actively steering away from it. The highest percentage occurred when L2 was paired with a live locust in Treatment 2 (72%, n = 13). However, when L2 was below L1, active steering was the least frequent type of response (17%, n = 3). When L2 was above L1, both active steering and non-directional startle responses occurred at similar frequencies and were the most prominent. Moreover, a non-directional startle response was the second most frequently

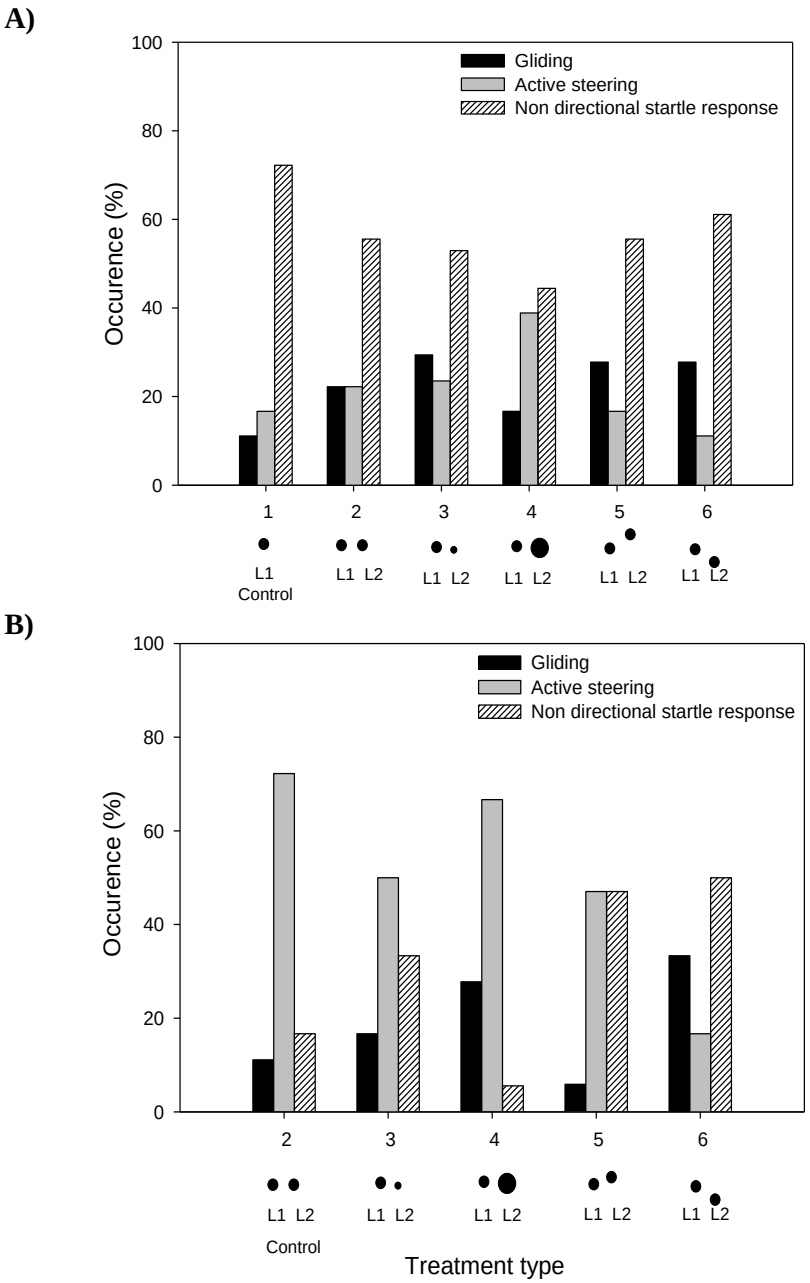


Fig. 3.9: Percentage occurrence of three responses; gliding, active steering and non-directional startle response of (A) L1 (*n*=18) and (B) L2 (*n*=18) in response to looming stimuli. Each column is the percentage of number of trials in which a particular type of behaviour was evident. Two black circles in each treatment are used to indicate the locusts' position. Relatively small and large circles in Treatments 3 and 4 show that L2 is in front of or behind L1, respectively. L1: Locust 1; L2: Locust 2.

occurring behaviour in Treatments 2 and 3, but was the most frequent response type in Treatment 6. However, it had the lowest frequency in Treatment 4 (6%, $n = 1$).

Of the three responses, gliding occurred least frequently in Treatment 2 (11%, $n = 2$), 3 (17%, $n = 3$) and 5 (6%, $n = 1$) whereas it had the second highest frequency in Treatments 4 and 6. These findings suggest that the most common response for L1 was non-directional startle and it can depend on the presence or different relative positions of a conspecific in the group. Conversely, L2 tended to avoid the stimulus by actively steering away from it, and by non-directional startle response in Treatment 6.

3.2.2 Direction of initial avoidance responses and extent of deviation along three translational degrees of freedom.

As illustrated in Figures 3.10A and B, despite the presence and different relative positions of L2, L1 flight paths in all the treatments were confined to a narrow range along the x-axis (±75 mm). Rather than deviating along the x-axis, the majority of flight paths were directed forward. This implies a smaller lateral deviation of L1 during collision avoidance. L2 flight paths, compared to that of L1, extended over a wider range along the x- axis (±100 mm), implying a greater lateral movement to the left and right directions in response to the looming stimulus.

Compared to the lateral deviation, L1 and L2 movement along the z-axis followed a similar trend. Both locusts had greater forward movements (+200 mm) and relatively smaller backward movements (-50 mm) in different treatments. Overall, L1 showed greater forward movements when it was alone in Treatment 1 and above L2 in Treatment 6. Greater forward movements were displayed by L2, when it was parallel to, in front of, or below L1 in

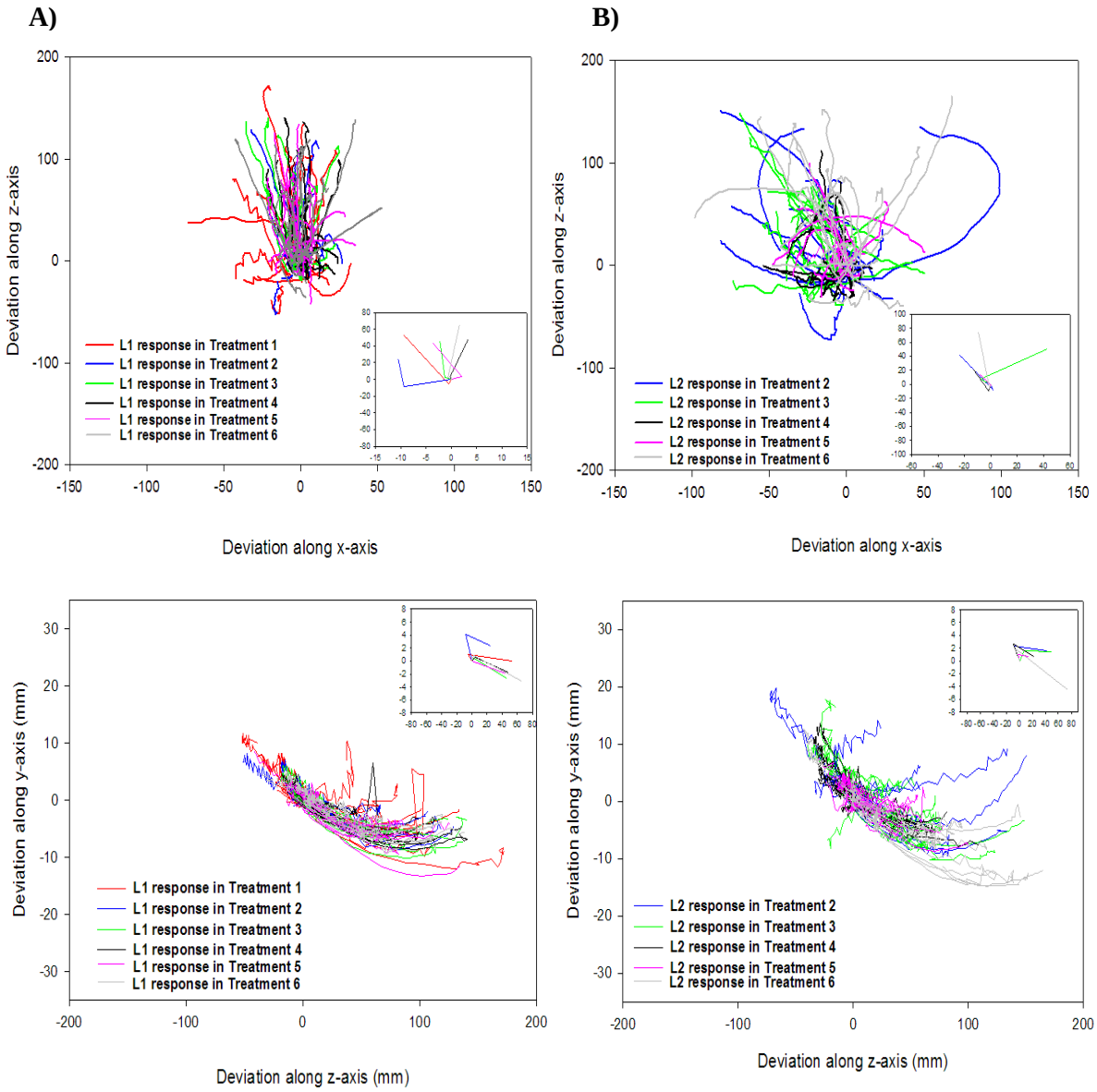


Fig. 3.10: Summary of the flight paths during initial avoidance responses taken by L1 (*n*=18) and L2 (*n*=18), in response to the laterally looming stimuli in the wind tunnel. A) Top panel: L1 deviation of movement along the x-axis vs. deviation along the z-axis as if viewed from above the wind tunnel. Bottom panel: L1 deviation of the movement along the z-axis vs. deviation along the y-axis as if viewed from the side of the wind tunnel. B) Top panel: L2 deviation of movement along x and z axes as if viewed from above the wind tunnel. Bottom panel: L2 deviation of movement along z and y axes as if viewed from side of the wind tunnel. Inset in each plot demonstrates the direction and average deviation of initial collision avoidance responses of L1 and L2 in each treatment. Greater average deviations in the motions of L1 and L2 are along the z-axis (+80). L1 average deviations along x-axis (±15) are low compared to that of L2 (± 60). Compared to x and z, average movements of both locusts along y-axis is low (±8). Relative to the initial position at the onset of the recording (zero), positive and negative deviations along the x-axis respectively, represent the rightward and leftward movements during the initial avoidance response. Whereas, the positive and negative deviations along the z-axis represent the forward and backward movements. Also, upward movements are positive and downward movements are negative along the y-axis. L1: Locust 1; L2: Locust 2.

Treatments 2, 3 and 6. In addition, the locusts' movements along the y-axis displayed a similar trend. Overall, despite few instances of backward motion, forward movements along the z-axis caused a gradual decrease in the locusts' elevations in the y-axis. Additionally, most of these declines in elevation were followed by slight increments towards the end of the initial response. However, compared to the deviation along the x- and z-axes, locusts' movements along the y-axis were relatively low.

Three translational degrees of freedom were compared between each treatment and with that of the control (Fig. 3.11). Comparisons were done to determine whether collision avoidance behaviour of L1 was affected by the relative position of a conspecific. L1 behaviour in different treatments significantly differed from that of the control. When L2 was below L1, significant changes in total sideslip translation (Tukey Test, $n = 18$, $H_5 = 12.720$, $P = 0.026$) and left movements (Dunn's Method, $n = 18$, $H_5 = 12.218$, $P = 0.032$) were detected (Fig. 3.11A). Relative to the control, both responses were reversed and more variable. Furthermore, upward movement of L1 was significantly affected when L2 was in front of L1 (Tukey Test, $n = 18$, $H_5 = 13.452$, $P = 0.019$) (Fig. 3.11A). Although, the position of L2 did not affect the forward and backward movement of L1, it affected the total thrust translation of L1 when L2 was parallel to, in front of, or above L1 (Tukey Test, $n = 18$, $H_5 = 15.709$, $P = 0.008$) (Fig. 3.11A). Relative to the initial response in the control, total thrust translation was significantly low.

L2 behaviour was compared between each treatment and with that of the control. Comparisons were done to determine whether behaviour depended on the position of L2 within the pair (Fig. 3.11B). Back, higher and lower positions in Treatments 4, 5 and 6, respectively, elicited comparatively weaker responses in sideslip movements (left and right). In these treatments, the total movement was significantly lower (Tukey Test, $n = 18$, $H_4 = 15.676$, $P = 0.003$) than that of the control treatment in which L2 was parallel to L1 (Fig.

3.11B). Total lift movement of L2 was significantly affected when it was above L1 in Treatment 5 (Tukey Test, $n = 18$, $H_4 = 9.991$, $P = 0.04$). Also, downward movement of L2 in Treatment 6 differed significantly from that of Treatment 5 (Tukey Test, $n = 18$, $H_4 = 15.431$, $P = 0.004$) (Fig. 3.11B).

Front and higher positions of L2 significantly affected its own thrust movement, compared to that of the control (Fig. 3.11B). In Treatment 3, total thrust was reversed and significantly higher with greater variability. However, it was significantly lower and less variable in Treatment 5 (Tukey Test, $n = 18$, $H_4 = 14.486$, $P = 0.006$). Compared to the control, there was no significant difference in forward movement in the other four treatments, but L2 forward movement in Treatment 6 was significantly different from that of Treatments 3 and 5 (Tukey Test, $n = 18$, $H_4 = 13.452$, $P = 0.009$). Significant differences from the control were also found in backward movement of L2, when it was in front of and below L1 in Treatments 3 and 6 (Tukey Test, $n = 18$, $H_4 = 11.328$, $P = 0.023$). These findings suggest that a locust's three translational degrees of freedom in initial avoidance response are significantly affected by the relative position of a conspecific, as well as its own position within the group.

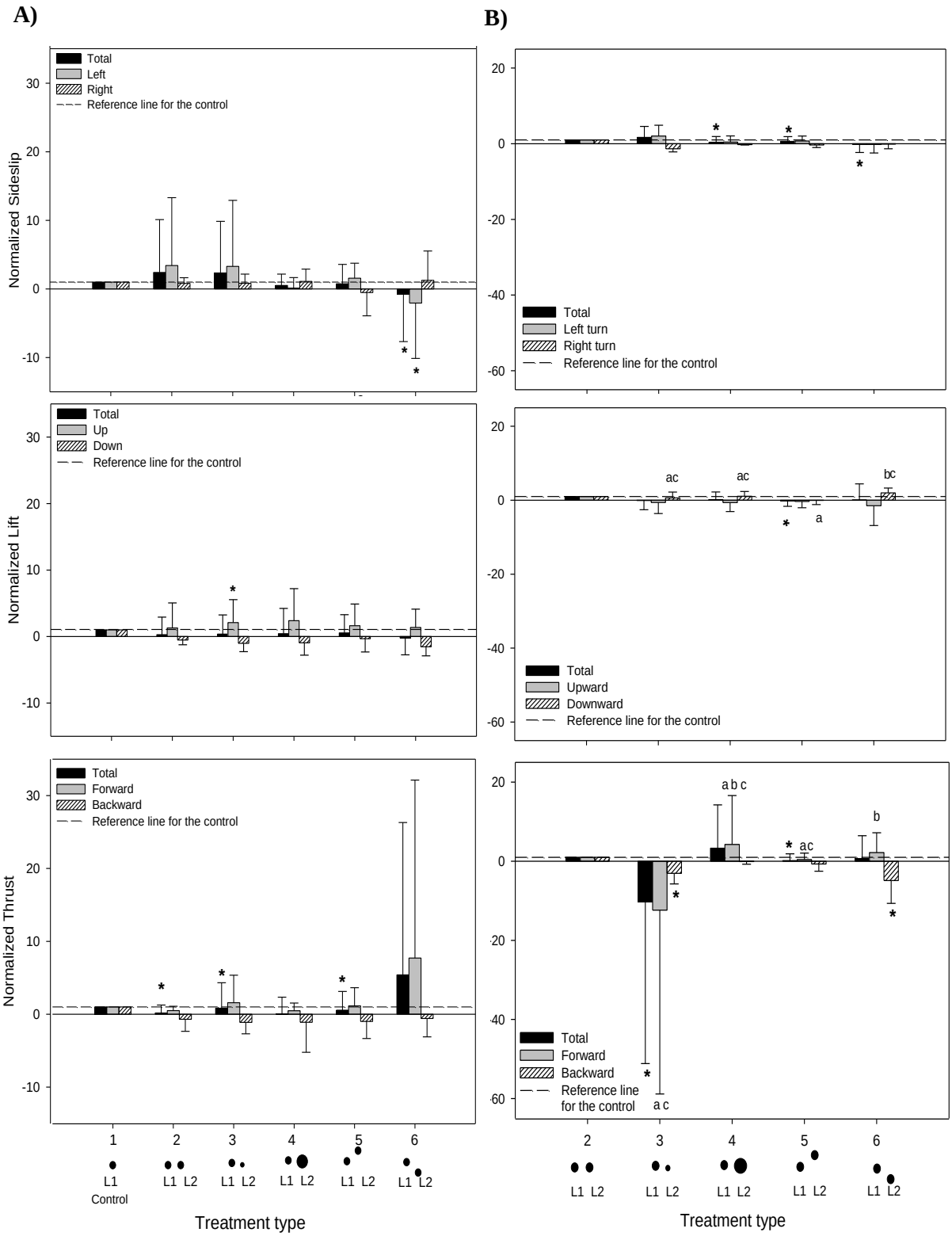


Fig. 3.11: A) Statistical summary of direction of the initial avoidance response and normalized extent of deviation of three translational degrees of freedom of L1 (n=18) and B) L2 (n=18). Two black circles in each treatment are used to indicate the locusts' position in each treatment. Relatively small and large circles in Treatments 3 and 4 indicate that L2 is in front of and behind L1, respectively. Error bars represent the standard deviation. Comparisons were made between treatments and asterisks indicate significant differences with the control at $P < 0.05$. Treatments with similar letters indicate no significant differences. L1: Locust1; L2: Locust 2.

3.2.3 Timing of the initial avoidance response relative to projected collision

Timing of the initial avoidance response of L1 (n = 18) and L2 (n = 18) in each treatment was compared to determine if there was an effect of the relative position of a conspecific. Figure 3.12A shows that the median time of L1 response onset in the control occurred 837 ms before the projected time of collision. Compared to the control, L1 responded 14, 18, 17, 26 and 9 ms (median time of the response onset) earlier in Treatments 2-6, respectively. However, there were no statistically significant differences between treatments, or compared to the control (Fig. 3.12A). L1 responded to the looming object almost at the same time during the six treatments, indicating that the time of the initial avoidance response of L1 was unaffected by the relative position of a conspecific in its vicinity.

The median time of L2 response onset in the control, occurred 849 ms before the projected time of collision (Fig. 3.12B). Compared to the control, the median time of L2 response onset was 86, 105 and 52 ms earlier when it was in front of, above and below L1 in Treatments 3, 5 and 6, whereas it was 11 ms later in Treatment 4. In Treatment 5, the initial avoidance response onset occurred significantly earlier than that of the control (Tukey Test, n = 18, H_4 = 13.261, P = 0.01, Fig. 3.12B). Significant differences were also identified between Treatments 4 and 5. These results suggest that the presence of a live conspecific at different relative positions does not affect the timing of the initial response onset of a locust in a group. According to L2 avoidance behaviour, a higher position in the group could evoke avoidance responses relatively earlier. This implies that a locust's own position within a group affects timing onset of its collision avoidance behaviour.

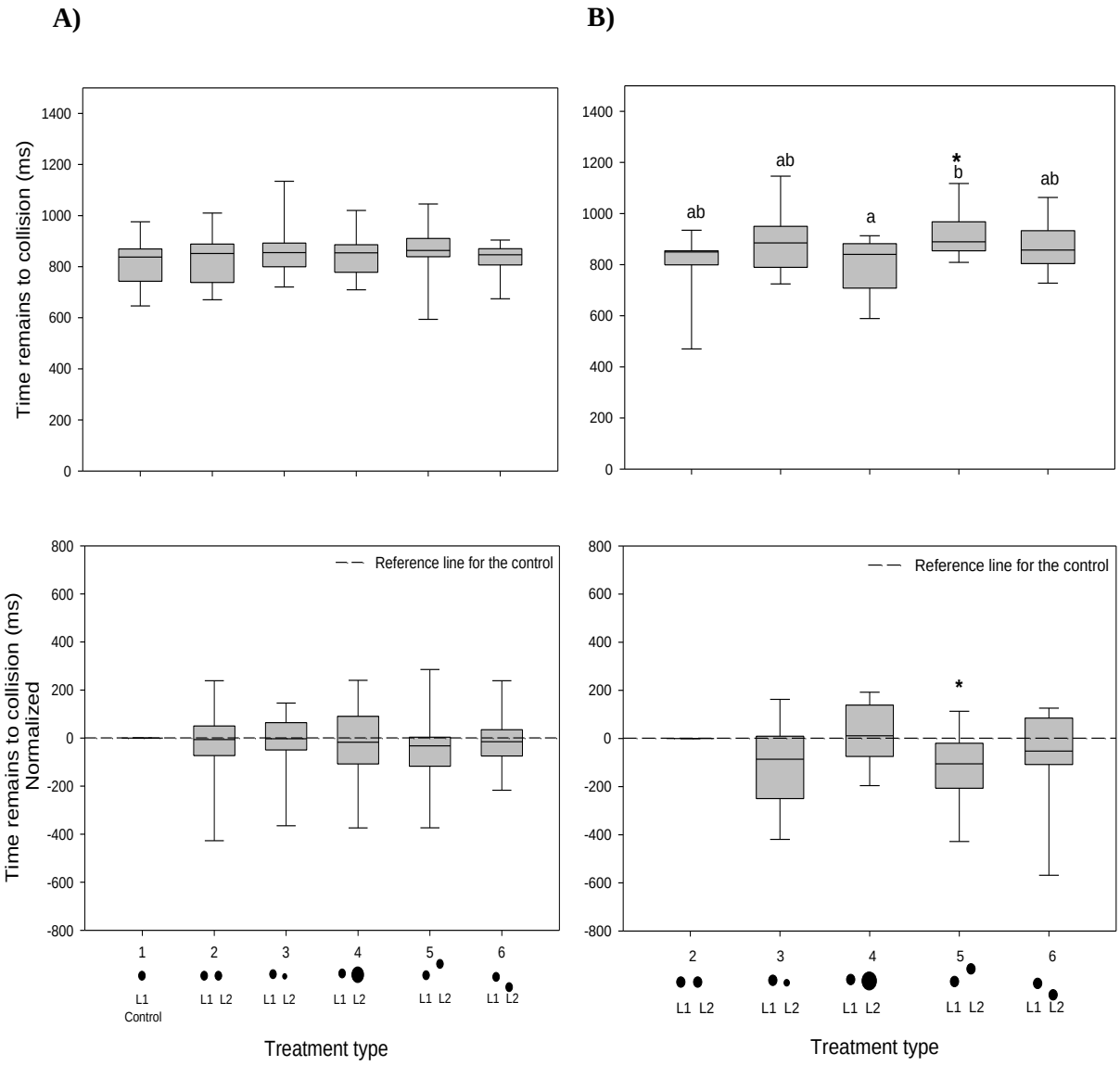


Fig. 3.12: Effect of treatment on onset of initial avoidance response of A) L1 (n=18) and B) L2 (n =18). Difference between the time of initial response and projected collision indicates the time remaining to collision. Thus, a greater difference between treatments indicates earlier response onset. The 1st and 2nd panels indicate the raw and normalized time values, respectively. Two black circles in each treatment are used to indicate the locusts' position in each treatment. Relatively small and large circles in Treatments 3 and 4 show that L2 was in front and behind L1, respectively. Boxes show the 25th and 75th percentiles and median value and whiskers show the 5th and 95th percentile level. An asterisk indicates the significant difference from the reference value (dashed line) at $P < 0.05$. Treatments with similar letters indicate no significant differences. L1: Locust 1; L2: Locust 2.

3.2.4 Duration of the initial avoidance response

The duration of the initial avoidance response was measured to determine if it was affected by the relative position of a conspecific. As illustrated in Figure 3.13A, the duration of the initial response of L1 in the five treatments was slightly lower than in the control. However, there were no significant differences between treatments or compared to the control. The lack of significant differences suggests that the relative position of a conspecific does not affect the response duration or response speed of a locust in a group.

L2 avoidance response duration in five treatments was slightly higher compared to that of L1. This suggests a slower reaction of L2 relative to L1 (Fig. 3.13A and B). Compared to the L2 behaviour in the control, response duration increased within the range of 58-110 ms when L2 was at different relative positions in the group (Fig. 3.13B). However, significant differences were not identified between the treatments or compared to the control. Lack of significant differences in the avoidance response duration suggest that a locust position within the group does not have a significant impact on its own reaction speed.

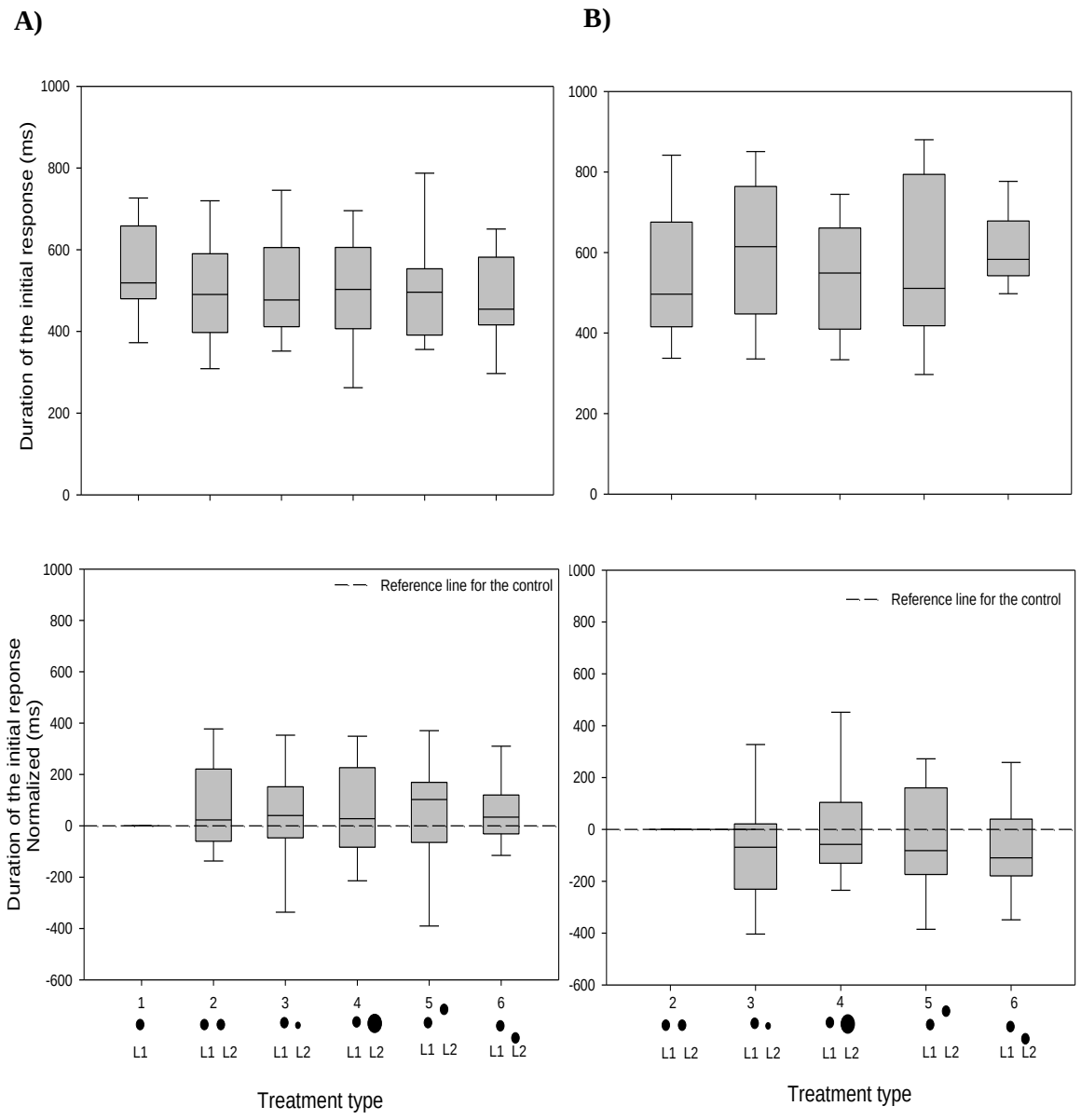


Fig. 3.13: Effect of treatment on initial avoidance response duration of A) L1 (*n*=18) and B) L2 (*n* =18). A difference between the onset and peak of the initial response was considered as the duration of the initial response. The 1st and 2nd panels indicate the raw and normalized time values, respectively. Two black circles in each treatment are used to indicate the locusts' position in each treatment. Relatively small and large circle in Treatments 3 and 4 shows that L2 is in front and behind L1, respectively. Boxes show the 25th and 75th percentiles and median value and whiskers show the 5th and 95th percentile level. L1: Locust1; L2: Locust 2.

3.2.5 Direction and distance between each locust at the time of the peak response

At the beginning of the experiment, the distance between two locusts along the x-axis was 30 cm. Locusts maintained the distance at the onset of the avoidance response fairly close to 30 cm (Table 3.1). Also, there were no significant differences in the L1-L2 distance at the onset of the response, between each treatment and compared to the control. Lack of significant differences between treatments suggest that, regardless of the presence of a conspecific at different relative positions, a locust pair can maintain the distance between each other during flight.

However, the distance between the pair at the peak of the response differed with treatments significantly. The mean distance was least in Treatment 3 and this was significantly different from that of Treatment 5. The distance between the two locusts was highest (31 cm) in Treatments 5 and 6.

Table 3.1: Effect of relative position of a conspecific in a group on the probability of steering to the same direction

Treatment number	Mean distance between L1 and L2 at the onset of the response (cm)	Mean distance between L1 and L2 at the peak of the response (cm)	Number of trials in which locusts moved in the same direction (n)	Probability % (N=18)
2	$31.8^{a} \pm 1.8$	$29.4^{ac} \pm 3.2$	11	61.1
3	$31.3^{a} \pm 1.7$	$27.7^{a} \pm 3.0$	9	50.0
4	$32.3^{a} \pm 1.9$	$30.7^{ac} \pm 2.0$	10	55.5
5	$31.2^{a} \pm 1.5$	$31.0^{bc} \pm 2.3$	12	66.7
6	$32.0^{a} \pm 3.5$	$31.0^{ac} \pm 4.6$	12	66.7

Comparisons made between treatments with similar letters indicating no significant differences at $P < 0.05$.

As illustrated in Table 3.1, in over 50% of trials, both locusts flew in the same direction simultaneously, while others flew in the opposite direction (i.e., either towards or from each other) during the initial avoidance response. I observed a higher probability of steering to the same direction when conspecifics were at relatively higher and lower positions in Treatments 5 and 6. Also, the greater probability resulted in a greater distance between two locusts at the peak. These findings suggest that, though the conspecifics remain at different relative positions, locusts are able to respond to the looming object as a pair.

4. DISCUSSION

Individual locust collision avoidance behaviour in response to looming stimuli has been studied previously (Robertson and Reye, 1992; Robertson and Johnson, 1993a, 1993b; Gray et al., 2001; Gray, 2005; Santer et al., 2005; Santer et al., 2006; Fotowat and Gabbiani, 2007; Simmons et al., 2010). However, no studies have investigated the collision avoidance behaviour of an insect/locust with a conspecific in the vicinity. This is the first study to test collision avoidance behaviour in a pair of flying locusts. The current study hypothesized that collision avoidance behaviour of a locust is affected by the presence and relative position of a conspecific. The objective of this study was to describe collision avoidance behaviour in a pair of flying locusts by examining different spatiotemporal characteristics and their dependency on the presence, as well as different relative positions, of a conspecific.

Supporting the hypotheses, the types of collision avoidance responses and some components of six degrees of freedom of L1 and L2 in Experiment 1 were affected by the presence of a conspecific in the vicinity. The types of avoidance responses and three translational degrees of freedom were also affected by the relative position of the conspecific. Also, we found that the timing of the onset and duration of the initial avoidance response of L2 were affected by the presence of a conspecific as well as its own position in the wind tunnel. Moreover, both locusts' responses to the looming stimuli were more robust in the presence of a live conspecific and less pronounced in the presence of a dead locust. According to the results of the current study, a locust's collision avoidance behaviour can be affected by the presence as well as relative position of a conspecific in the vicinity.

4.1 Experimental paradigm

Locusts have been used as a model to study different aspects of collision avoidance behaviour. Examples include flight steering (Robertson and Johnson, 1993a; Santer et al., 2005), wing kinematics (Robertson and Reye, 1992), motor pattern in flight muscles (Santer et al., 2005), sensory coding and visual processing during collision avoidance (Judge and Rind, 1997; Rogers et al., 2010). However, these studies examined the behaviour of a single locust. While Camhi et al. (1995) described wing beat coupling between a pair of rigidly tethered locusts, they did not examine collision avoidance. Therefore, the use of two loosely tethered locusts in the present study is a new approach to understanding 3D looming-evoked behaviour with a conspecific in the vicinity.

Gregarious locusts aggregate into swarms in the wild. A swarm may contain 10^{10} individuals that may be flying along the same or different trajectories at different velocities. A swarm includes sub-clusters of many individuals flying together (Baker et al., 1984). Thus, while studying a pair of locusts may represent only a crude approximation of natural conditions, it is a first attempt to study dynamics of conspecific flight behaviour within the physical constraints of a controlled experimental set up. In previous studies, locusts have often been rigidly held in a wind tunnel to facilitate behavioural and physiological recordings. This rigid tethering introduces artefacts since the locust is unable to evoke complete steering behaviours (Spork and Preiss, 1993; Santer et al., 2005). Moreover, this can cause steering biases in the motor pattern that controls wing beat and results in generation of insufficient lift. The use of loose tethers in the present study permits freedom of movement within 3D space and thus better reflects the initial natural flight steering responses.

4.2 Types of initial avoidance responses

Visually evoked collision avoidance has been studied across different animal species including locusts (Gabbiani et al., 1999; Gray et al., 2001; Santer et al., 2005), crabs (Oliva et al., 2007), flies (Sugiura and Dickinson, 2009), frogs (Nakagawa and Hongjian, 2010), gerbils (Ellard, 2004), birds (Sun and Frost, 1998; Cao et al., 2004), fish (Preuss et al., 2006) and monkeys (Schiff et al., 1962; Maier et al., 2004). The size of the retinal image subtended by the approaching object is an important stimulus parameter in triggering visually evoked avoidance responses. However, the subtense angle that evokes collision avoidance behaviour, can vary among different animal systems: 30-35˚ in fiddler crabs and chicks (Kang and Li, 2010), 21.1˚ in frogs (Nakagawa and Hongjian, 2010) and ~10˚ in tethered migratory locusts (Robertson and Johnson, 1993a). In the present study, at its final position on the screen, the looming stimulus subtends 6.5˚ and 13.0˚ of the visual field of view of L1 and L2, respectively. Since both L1 and L2 were able to respond to the looming stimulus during its approach, the results of this study suggest that the locusts are capable of initiating an avoidance reaction when the stimulus subtends less than 10˚ of the visual field.

The looming evoked collision avoidance responses observed in each locust in the present study are consistent with that of previous behavioural studies (Robertson and Reye, 1992; Robertson and Johnson, 1993a; 1993b; Gray et al., 2001; Santer et al., 2005). Active steering was the most prominent type of avoidance response exhibited by L1 and L2 in the presence of a dead and a live locust in Experiment 1 (Fig. 3.2). Also, when at different relative positions in Experiment 2, L2 responded to the looming stimulus most frequently by actively steering away from it (Fig. 3.9). Conversely, L1 displayed a non-directional startle response and gliding as the most prominent and second most frequent behaviours, respectively, in Experiment 2.

The characteristics of active steering seen in this study, namely abdominal flexion and hind leg extension into the turn, are consistent with that of previous studies (Robertson and Johnson, 1993a; 1993b; Santer et al., 2005). Presentation of different targets towards the head of a locust on a collision course triggered steering behaviours which directed the animal around an obstacle in its path (Robertson and Reye, 1992). These characteristics were also observed in thermal and auditory avoidance behaviours (Robertson et al., 1996; Dawson et al., 1997). Consistent with prior observations, characteristics of active steering in locusts in the present study can be interpreted as an attempt to move away from the looming stimuli. Walk in to the opposite direction during collision avoidance has also been found in crabs responding to laterally looming stimuli (Lindemann et al., 2008). A fruit fly (*Drosophila*) exhibits sharp fast turns commonly referred to as saccades, and rapidly expanding objects from one direction in a fly's visual environment trigger a saccade in the opposite direction (Frye and Dickinson, 2004). When human infants were presented with approaching objects, they tended to move their head back and away from the object (Ball and Tronick, 1971).

When an animal encounters an approaching object, it will experience an increase in retinal image size of the object independent of background motion (Verspui and Gray, 2009). Frequent active steering in both locusts irrespective of the presence of a dead or a live conspecific suggests that looming stimuli with increasing retinal image size represent more acute danger and gain more attention than the stationary dead conspecific or live conspecific exhibiting translatory movement in the lateral visual field (Dukas, 2002; Verspui and Gray, 2009). In schooling fish (e.g., herring), solitary individuals display different, variable, escape trajectories compared to members of a group, which display relatively uniform escape trajectories (Domenici and Batty, 1997; Domenici et al., 2011). In accordance with this previous study, consistency in the type of response in both locusts when they were in close proximity in Treatment 5 in Experiment1 suggests that locusts use visual cues from

immediate neighbours and that the near presence of a conspecific can affect the collision avoidance behaviour of a locust. The lack of consistency in response types between L1 and L2 in Experiment 2 cannot, however, be attributed to a conspecific effect.

Active steering in collision avoidance is triggered when an image of a looming stimulus reaches a fixed angular subtends at the eye. If the stimulus is detected late during looming, the same stimulus results in gliding behaviour or a non-directional startle response (Robertson and Reye, 1992; Gray et al., 2001). Thus, it is likely that gliding and non-directional startle responses of L1 in Experiment 2 were due to the late detection of the looming object. The presence of L2 at different relative positions may have affected L1's visual sensory perception. Moreover, consistency in L1 avoidance response across all treatments would further suggest that L1 avoidance behaviour is affected by the presence of a conspecific at different relative positions and may not depend on a specific position. As noted by Gray et al. (2001), locusts showed startle behaviour in response to a 10 cm diameter sphere approaching in converging, offset and diverging trajectories in the frontal field of view. This was interpreted as a late detection of the fast looming stimulus and thus a failure to make an effective flight steering response. In addition, gliding behaviour in a tethered locust has also been noted previously in response to the late detection of faster approaching binocular head-on stimuli (Robertson and Reye, 1992) or a laterally looming disc (Santer et al., 2005). According to previous reports, in free flying locusts, looming-elicited gliding appears to be an emergency response suited to the evasion of fast aerial predators. Gliding and startle responses evolved primarily as an escape mechanism from approaching predators in different animal species such as flying squirrels (Paskins et al., 2007), bush crickets (Libersat and Hoy, 1991), great and blue tits (Kullberg et al., 1998; Lind et al., 2002) and mantis (Maldonado, 1970; Yamawaki and Toh, 2009). However, an increase in gliding response frequency by L1 in Experiment 2, compared to that of Experiment 1 suggests that

locusts use visual cues from the looming objects as well as from flying neighbours during collision avoidance.

My observations of wing folding after a glide and non-directional startle responses, rather than resumption of flight, are consistent with previous findings (Gray et al., 2001; Santer et al., 2005). It is believed that flight cessation after these behaviours would be an artefact of tethering. Under natural conditions, flight resumption after gliding and non-directional startle responses are more likely to occur than under experimental conditions. This is because the height loss during these behaviours increases air flow over the head and excites sensory hairs that trigger wing depressor motor neurons and in turn resumes flight by depressor muscle contractions (Pond, 1972; Simmons, 1980). Conceivably, lack of such sensory information could eventually cause flight cessation in tethered flight. The results of this study suggest that locust collision avoidance can vary in response to the same looming stimulus depending on the presence and relative position of a conspecific. Unpredictability in escape trajectory may prevent a predator learning a simple escape pattern and result in lower vulnerability to attack in the wild.

4.3 Responses within translational and rotational degrees of freedom

Changes in the translatory pattern of motion in the locust's lateral visual field simulates changes in flight speed (thrust) and flight direction (yaw) (Spork and Preiss 1993). Thus, the speed and direction of locust flight in a swarm results from the optomotor effectiveness of the pattern image formed by the neighbouring individuals and this feature contributes to a common orientation within a group. In contrast, the current study showed that L1 generated significant sideslip irrespective of the presence of a live or dead locust. However, significant changes in the thrust translation of L1 in Experiment 1 (Fig. 3.4) are consistent with the findings of Spork and Preiss (1993). Significantly increased or decreased

forward movements when coupled with a live or dead locust, respectively, showed that dead or live conspecifics have different effects on a flying neighbour's collision response. Thus, translational degrees of freedom in collision avoidance behaviour of L1 can be affected by the presence of a conspecific in the vicinity.

Previous studies have shown that in general, an animal's response to small translating objects is less pronounced compared to a response to an approaching object (Nakagawa and Hongjian, 2010). Two movement detector neurons in the crab (*Chasmagnathus granulates*) exhibited more robust responses to a black looming stimulus, whereas their responses to lateral displacement of a 6 cm black square were comparatively low (Oliva et al., 2007). Similarly, collision sensitive neurons in the frog (*Rana catesbeiana*) also showed a much stronger response to looming stimuli than to translating objects in the animal's visual field (Nakagawa and Hongjian, 2010). According to Oliva et al. (2007), crabs display sideslip movements in the opposite direction to a laterally expanding stimulus and the escape paths in response to lateral stimuli are longer than those of a frontal or dorsal stimulus. Similarly, tethered fruit flies (*Drosophila*) are capable of generating side-slip force and roll away from the laterally expanding stimulus in a flight arena and both reactions accelerate the animal away from the expanding stimulus (Sugiura and Dickinson, 2009). In accordance with previous studies, L2 showed robust responses consisted with frequent active steering and greater sideslip movements (Fig. 3.3), and more often flew in the opposite direction to the looming stimulus. However, compared to the control, there were no significant deviations in L2 sideslip, when paired with a live or a dead locust. This suggests that there is a greater looming effect over the conspecific effect on L2's avoidance behaviour in Experiment 1. However, deviations in the three translational degrees of freedom depend on the type of avoidance strategy taken by locusts in flight. According to Wilson and Weis-Fogh (1962), flying locusts appear to regulate lift independent of thrust and have also been claimed to

exhibit constant lift following imposed changes of body angle up to 20°. However, during steering, lift is reduced on the side of the yaw turn and this is often accompanied by diminution of thrust and increase in sideslip (Baker, 1979). Accordingly, significant differences in both locusts' lift translation in Experiments 1 and 2 can be related to the presence of different avoidance responses and thus can be attributed to the presence and different relative positions of the conspecific, respectively. Compared to the control, significant differences in L2 thrust translation in Experiment1 could also be a result of the presence of a conspecific (Fig. 3.4).

Deviations were also found in L1 and L2 sideslip and thrust translation in Experiment 2 (Fig. 3.12). Results suggest that translational degrees of freedom of L1 and L2 in Experiment 2 are affected by the presence of a conspecific at different positions and its own position, respectively. There were significant differences in thrust translation of L2 in Treatments 3, 5 and 6 where L2 was in front of, above and below L1 in Experiment 2. Compared to the control, significantly low total sideslip translation was also found in Treatments 4, 5 and 6. In the current study, looming stimuli expanded along a trajectory perpendicular to the longitudinal axis of the locusts. Thus, in the control/Treatment 2, the centre of the looming object loomed directly towards the centre of the right eye of the locusts. Accordingly, compared to the control, in Treatments 3 to 6, L2 may have experienced the looming stimulus across a broad region of the visual field. Thus, significant changes in thrust and sideslip translations in different treatments, may be due to stimulation of different regions of the locust's visual field. It has been previously reported that the locust's peripheral visual field is less sensitive in all directions relative to the broad lateral region (Rogers et al., 2010). Directional selectivity has also been found in other animal systems**:** collision sensitive neurons in the optic tectum of pigeons (Wylie and Frost, 1999), lobula plate interneurons in many fly species (Krapp et al., 1998) and neurons in the optic tectum of frogs (Kang and Li,

2010). Collision sensitive neurons in the frog possess smaller receptive fields than those of a locust and are activated only when the focus of expansion of a looming retinal image is located within the centre of its receptive field (Kang and Li, 2010). The locust visual system contains two inter-neurons, the LGMD and its postsynaptic partner, the DCMD, that are highly responsive to looming stimuli and thought to be involved in fast escape behaviours (Simmons and Rind, 1992, Judge and Rind, 1997). This system, which is involved in triggering collision avoidance reactions, has high sensitivity to looming stimuli across a broad region of visual field, extending from 30˚ to 150˚ azimuth and from -15˚ to 45˚ elevation (Rogers et al., 2010). The DCMD is less sensitive to stimuli presented from below the eye equator than from above (Rind and Simmons, 1997; Guest and Gray, 2006; Rogers et al., 2010). The highest DCMD activity in response to local motion objects was found for stimuli presented in the posterior region of the visual field, whereas the presentations in the frontal region triggered comparatively less robust responses (Krapp and Gabbiani, 2005). Similarly, Guest and Gray (2006) found greater DCMD activity in gregarious locusts during individual approaches of a 7 cm diameter disc from 135˚ than approaches from 45˚. Apparently**,** these findings would account for the discrepancy in L2 behaviours in different treatments. On the other hand, significant leftward movement in L1 may be due to the higher sensitivity for the looming object approaching along the eye equator than the conspecific flying below in Treatment 6 (Rogers et al., 2010). Accordingly, decreased total thrust translation of L1 in Treatments 2, 3 and 5 can be attributed as the effect of the presence of a conspecific within a more sensitive region of the visual field of L1.

According to Robertson and Johnson (1993a), an obstacle approaching in a flight path of a tethered locust triggered steering behaviours associated with changes in yaw torque. They observed a maximum yaw torque to the left as an attempt to steer to the left when the target was on the right. Tethered *Drosophila* responded to lateral expansion of an object with

a change in both roll and yaw rotations while maintaining nearly constant pitch (Sugiura and Dickinson, 2009). Similarly, in the present study locusts changed the direction of their flight in response to the looming object by generating yaw torques or roll torques or both**,** while pitch remained nearly constant. In free flying locusts, angular changes during yaw and roll movements are closely related and angle values change in parallel almost simultaneously (Berger and Kutsch, 2003). However, in our experiments, these angular changes were neither consistent nor related. Significant changes in yaw and roll of both locusts (Fig. 3.5) imply that rotational degrees of freedom during collision avoidance can be affected by the presence of a conspecific. Contradictory patterns in the (reversed) total roll and right rotation deviations of L1 and (reversed) right rotation of L2: increased and decreased rotations when coupled with a live and dead locust, respectively, are consistent with findings of Spork and Preiss (1993) and provide further evidence that locusts use visual cues from the looming object as well as from proximal conspecifics.

4.4 Timing onset and duration of the initial avoidance response

Locust DCMD peak-firing rate and the time and duration of the peak in response to a looming object depend on the object size and approach velocity (Guest and Gray, 2006). Similar to locust's DCMD activity, that of looming sensitive neurons in other species, notably mantis (Yamawaki and Toh, 2009), and bullfrog (Kang and Li, 2010), is dependent on the size and velocity of the looming object. Accordingly, use of the same looming stimulus with unvarying properties throughout the experiments may have affected the timing of L1 and L2 collision avoidance behaviour equally and independently. According to Spork and Preiss (1993), the pattern of motion in the lateral visual field of a locust is able to induce changes not only in the amplitude but also in the time course of the response. In the current study, the pattern of motion in the locust lateral visual field changed in different treatments.

However, despite the significant differences in type and magnitude of escape behaviours, the L1 timing onset of the initial response was remarkably consistent. Though changes in the time course of the locusts' responses were to be expected, lack of significant differences emphasizes that the timing onset of L1 depended neither on the presence nor on the relative position of a conspecific (Figs. 3.6 and 3.12). However, significantly early response onset of L2, when it was coupled with a live locust in Experiment 1 and when placed at a higher position in Experiment 2, suggests that L2 response onset is dependent on the presence of a conspecific as well as its own position within the pair. On the other hand, lack of significant differences in L1 initial avoidance response duration in both experiments implies that response duration does not depend on the presence or different relative positions of a conspecific (Figs. 3.7 and 3.13). The results further suggest that L2 response duration under different treatment conditions is also not dependent on the presence of a conspecific and its own position in the pair. Consistency in the response duration in each locust would be due to the consistency in the time and duration of DCMD activity in response to the same looming stimulus.

L1 and L2 behaviour, in terms of onset and duration of the initial avoidance response, was contradictory between experiments. The LGMD responds to objects approaching on a direct collision course and produces its peak firing rate at a fixed delay after the looming object reaches a fixed angular size on the retina (Gabbiani et al., 1999; Gabbiani et al., 2002; Gray, 2005; Guest and Gray, 2006). Similarly, previous studies have shown that a looming-triggered jump in locusts and frogs occurs with a fixed delay after the looming stimulus reaches a fixed angular threshold size on the retina (Yamamoto et al., 2003; Fotowat and Gabbiani, 2007) and looming-elicited escape responses in *Drosophila* occur 22 ms after the looming stimulus reaches a threshold angular size of 47˚ (Fotowat et al., 2009). Another behavioural study showed that gerbils use changes in the retinal image size of the target to

detect the target distance (Ellard, 2004). Accordingly, gerbils can compute the time to collision and adjust their behaviour appropriately, namely, change the speed of movement. This study found slower running speeds or earlier braking in response to a larger retinal image size and conversely faster running or later braking in response to a smaller retinal image size. In the present study, the looming object would have reached a fixed angular size on the eye of L2 prior to that of L1, which was further away than L2. This would lead to an earlier response onset in L2 and conversely a later response onset in L1. Since very brief delays in response onset can have an impact on the speed and accuracy of the movement (Hu et al., 1999; Westwood et al., 2001), different response durations can be attributed to the discrepancy in the timing of response onset.

4.5 Distance between two locusts

The current study used gregarious locusts. Gregarious locusts flying in a swarm encounter many objects: conspecifics, predators and other flying animals, approaching frequently from many directions (Waloff, 1972; Uvarov, 1977; Rind and Santer, 2004; Gray, 2005). In a dense swarm, the conspecifics could be in different relative positions and may be flying along the same or different trajectories at different velocities. Despite the apparently random orientation of groups of individuals within the swarm, continuous cohesion of individual swarms over distances of hundreds of kilometres lasting many days has been observed without significant dispersion (Baker et al., 1984; Spork and Preiss, 1993). Locusts have evolved the ability to not only avoid predation, but also effectively navigate within the swarm without constantly colliding with one another (Baker et al., 1981). Flying locusts may also maintain their position relative to their immediate neighbours to some extent (Kennedy, 1951, Preiss, 1992). My findings provide evidence that two locusts flying very closely together, that is, 30 cm apart, can maintain this distance during flight (Fig. 3.8). Similarly,

schooling fish such as cod (*Gadus morhua*), saith (*Pollachius virens*) and herrings (*Clupea harengus*), have the ability to maintain the distance between the nearest neighbours within a set range (Parrish et al., 2002). According to Imada et al. (2010), when an optomotor response is elicited simultaneously in two conspecific puffer fish, they exhibit cohesive movement while maintaining a fixed distance.

Locusts can transfer directional information and rapidly change their flight direction appropriately (Baker et al., 1981; Farrow, 1990). Spork and Preiss (1993) suggested that the speed and direction of locust flight under free flight conditions are driven from the optomotor effectiveness of the image pattern formed by the neighbouring individuals and this eventually would contribute to swarm cohesion. In accordance with these findings, we found that in more than 50% of trials, locusts responded to the looming object as a pair. Moreover, in treatments that showed the higher percentage of pairing behaviour, locusts were able to maintain the distance during collision avoidance (Table 3.1). These findings further emphasize that regardless of the presence and/or different relative positions of a conspecific, locusts can maintain the distance from their neighbour and have the ability to respond to potential threats as a group.

4.6 General collision avoidance strategies.

In the current study both locusts responded to a looming stimulus with active steering, gliding or non-directional startle responses. These avoidance strategies consisted of motion away from the stimulus, across multiple trajectories in the horizontal plane. Therefore, under natural conditions, a locust's escape behaviour may not be confined to a single trajectory. Rather, a locust may exhibit multiple escape trajectories mostly within an 80-180˚ sector in the horizontal plane (Santer et al., 2005; Domenici et al., 2011). Moreover, gliding in free flight can result in escape trajectories in the vertical plane. Apparently, high variability in

locust escape trajectories across horizontal and vertical plane will make the escape response relatively unpredictable, which would counter learning by predators.

Multiple escape trajectories in response to a looming object have also been found in other animal species. *Drosophila* uses visual information to trigger a jump in a direction away from a looming threat (Card and Dickinson, 2008). Looming stimuli presented to the dorsal visual field of the crab (*Chasmagnathus granulates*) triggered escape trajectories toward the left or right and escape in a single direction away from a stimulus when presented in the horizontal plane (Oliva et al., 2007). When stimulated at 45˚ above the horizontal plane, cryptic frogs (*Craugstor*) escaped away from the stimulus at various angles relative to their body axis (Cooper et al., 2008). Among fish, guppies (*Poecilia reticulata*, Walker et al., 2005), and cowtail stingrays (*Pastinachus sephens*, Semeniuk and Dill, 2005) exhibit variable escape trajectories mostly within a 90-180˚ sector. Similarly, birds show a variety of escape trajectories, both in the horizontal and vertical planes. Based on the predator attack speed, some birds take off initially away from the predator and then climb back over the predator in vertical plane (e.g., great tits, *Parus major*; Kullberg et al., 1998, and blue tits, *Parus caerules*; Lind et al., 2002) or dart sideways approximately 90˚ from the predator's line of attack (sedge warbler, *Acrocephalus schoenobenus;* Kullberg et al., 2000). High variability in escape trajectories observed in different species may provide greater unpredictability in escape response and lower vulnerability to predator attack than does escaping at fixed angles. Many escape strategies exhibited by other animal models are similar to those of a locust. Thus, understanding the locust's avoidance behaviour under the present experimental context will provide insight into general avoidance strategies employed across species.

The results of the present study support the hypotheses that collision avoidance behaviour of a locust is affected by the presence as well as different relative position of a conspecific. Results further emphasize that locusts use visual cues from the looming object as

well as from an adjacent conspecific. Locust responses to looming stimuli were more robust in the presence of a live conspecific and less pronounced in the presence of a dead locust. The presence of a conspecific, irrespective of collision avoidance behaviour, may also influence the response of the first locust in the vicinity.

This behavioural study provides insights into collision avoidance behaviour in a pair of flying locusts in a swarm. Since I used only two locusts, further experimentation with a greater number of individuals will be required to extend these findings to more natural conditions. Also, studying general and specific interactions between flying locusts during collision avoidance behaviour will be crucial to understanding the physiological mechanisms of behaviour under more realistic natural conditions. Experiments using concurrent behavioural and neurophysiological techniques are required to understand the neural correlates underling collision avoidance behaviour in pair of flying locusts. Thus, future investigations should incorporate studies of general behaviour, wing kinematics, sensory coding and transformations to motor output to better understand the underlying neural mechanisms and coordinated and cohesive movement between individuals during the production of natural behaviour.

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